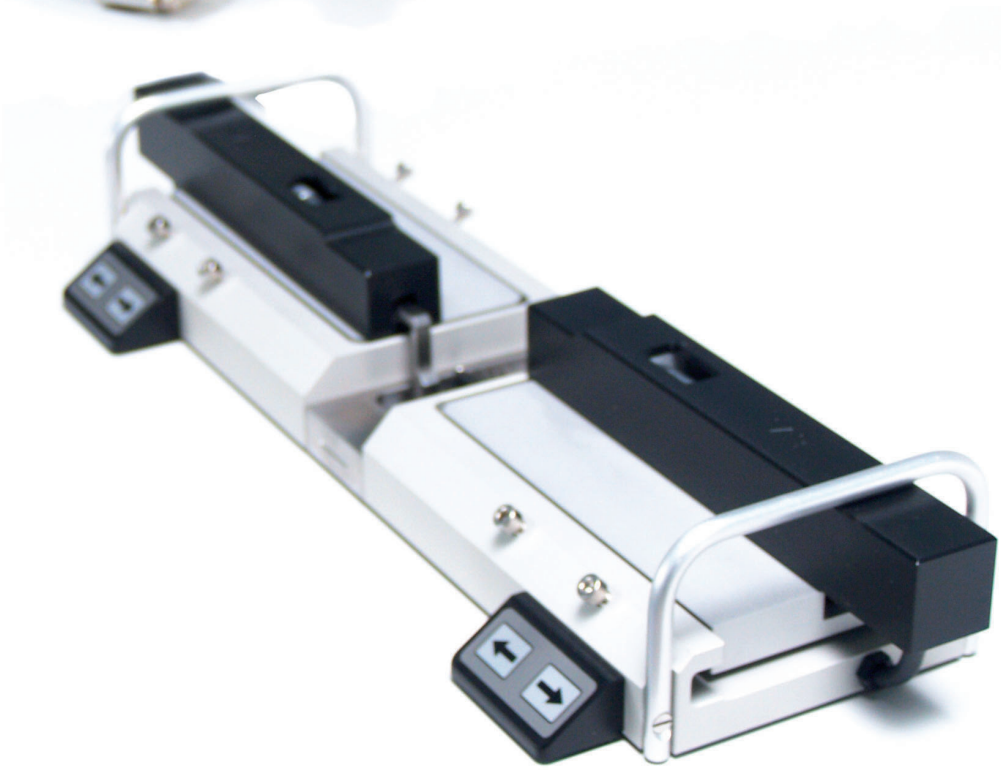
Auto Dual Wire Myograph System

Model 510A



User Manual

Version 3.3

DMT

DANISH MYO TECHNOLOGY

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Auto Dual Wire Myograph System - Model 510A

User manual

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This documentation is provided with the DMT Auto dual wire myograph system – Model 510A – v. 3.3

Document Number: 510A – UG3.3A

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Introduction

Until the mid-1970s most of the details about the mechanical, morphological and pharmacological properties of vascular smooth muscle was obtained from studies on relatively large vessels. At that time, rat-tail arteries were the smallest vessels to be investigated in detail due to limitations in the available *in vitro* techniques. For example, studies measuring the contraction force were routinely performed with only one of the mounting wires secured. Furthermore, relatively large wires (100-200 µm) were used, which precluded the use of small vessels, and the vessel segment had to be directly manipulated with the dissecting equipment causing inevitable mechanical trauma. Investigations of smaller vessels were therefore limited to *in vivo* perfusion experiments and histological examinations.

In 1976 Professor M. J. Mulvany and Professor W. Halpern described for the first time a new technique that made it possible to investigate highly isometric responses from vessels with internal diameters as small as 100 µm. The mounting procedure was refined twofold: both ends of each mounting wire were secured under tension without the need to manipulate the vessel segment. Segments of small vessels could now be atraumatically mounted as ring preparations in a myograph for recording of highly isometric force measurements.

During the late 1970s some improvements were made to the myograph and in 1981 a new dual myograph that allowed simultaneous testing of two vessels was introduced. In parallel, the technique became more acknowledged worldwide resulting in a growing interest in the myograph systems. In 1986, the growing demand resulted in the foundation of the private company J P Trading with the purpose of making the myograph systems commercially available worldwide. At the same time J P Trading initiated a comprehensive improvement programme for the existing myograph systems as well as a development programme of new myograph systems in close co-operation with Professor M. J. Mulvany and the University of Aarhus.

During the late 1980s and through the 1990s several improvements were applied to the myograph systems, such as a new mechanical design, a more robust transducer and new electronic systems. In addition, new systems were introduced, like the Automatic Dual Myograph 510A, the Dual Myograph 410A, the Multi Myograph 610M and the Confocal Myograph 120CW. In 2000 J P Trading changed its company structure and became known as DMT.

Today DMT is one of the world's leading designers and manufacturers of wire myographs, pressure myographs, culture myographs and organ/tissue baths. Driven by our global customer base, our number one goal is to develop and manufacture first class research equipment within the fields of physiology and pharmacology.

Safety

The Auto dual wire myograph system has been designed for use only in teaching and research applications. It is not intended for clinical or critical life-care use and should never be used for these purposes: nor for the prevention, diagnosis, curing, treatment, or alleviation of disease, injury, or handicap.

- Do not open the unit: the internal electronics pose a risk of electric shock.
- Do not use this apparatus near water.
- To reduce the risk of fire or electric shock, do not expose this apparatus to rain or moisture. Objects filled with liquids should not be placed on the apparatus.
- Do not block any ventilation openings. Install in accordance with the manufacturer's instructions.
- Do not install near any heat sources such as radiators, heat registers, stoves, or other apparatus that produce heat.
- Only use attachments and accessories specified by the manufacturer.
- Unplug this apparatus during lightning storms or when unused for long periods of time.
- This apparatus must be grounded.
- Use a three-wire grounding-type cord similar to the one supplied with the product.
- Do not defeat the safety purpose of the polarized or grounding-type plug. A polarized plug has two flat blades, one being wider than the other. A grounding type plug has two blades and a third (round) grounding pin. The wide blade or the third prong is provided for your safety. If the provided plug does not fit into your outlet, consult an electrician for replacement of the obsolete outlet.
- Be advised that different operating voltages require the use of different types of line cord and attachment plugs. Check the voltage in your area and use the correct type. See the table below:

Voltage	Line plug according to standard
110–125 V	UL817 and CSA C22.2 No. 42.
220–230 V	CEE 7 page VII, SR section 107-2-D1/IEC 83, page C4.
240 V	BS 1363 of 1984. Specification for 13A fused plugs and switched and unswitched socket outlets.

Protect the power cord from being walked on or pinched: particularly at power plugs and the point where they connect to the apparatus.

Refer all servicing to qualified service personnel. Servicing is required when the apparatus has been damaged in any way; such as, the power-supply cord or plug is damaged, liquid has spilled onto or objects have fallen into the apparatus, the apparatus has been exposed to rain or moisture, does not operate normally, or has been dropped.

EMC / EMI

This equipment has been tested and found to comply with the limits for a Class B Digital device, pursuant to part 15 of the FCC rules. These limits are designed to provide reasonable protection against harmful interference in residential installations. This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception (which can be determined by monitoring the interference while turning the equipment off and on), the user is encouraged to correct the interference by one or more of the following measures:

- Reorient or relocate the receiving antenna.
- Increase the separation between the equipment and receiver.
- Connect the equipment into an outlet on a circuit different to that which the receiver is connected to.
- Consult the dealer or an experienced radio/TV technician for help.

Approvals

Complies with the EMC standards:

EMC 89/336/EEC: EN 61326-2-6:2005
EN 61000-3-2.

Certified with the safety standards:

Directive 2006/95/EC: EN 61010-1:2001
EN 61010-1/Corr.1:2003
EN 61010-1/Corr.1:2003
EN 61010-2-101:2003

Certificate of Conformity

DMT A/S, Skejbyparken 152, 8200 Aarhus N., Denmark,
hereby declares its responsibility that the following product:

Auto Dual Wire Myograph System
Model 510A version 3.3

is covered by this certificate and marked with CE-label conforms with the following standards:

EN 61010-1:2001 EN61010-1/Corr.1:2003 EN 61010-1/Corr.1:2003	Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 1: General requirements.
EN 61010-2-101:2003	Safety requirements for electrical equipment for measurement, control and laboratory use - Part 2-101: Particular requirements for in vitro diagnostic (IVD) medical equipment.
EN 61326-2-6:2005	Electrical equipment for measurement, control and laboratory use - EMC requirements - Part 2-6: Particular requirements - In vitro diagnostic (IVD) medical equipment.
	With reference to regulations in the following directives: 2006/95/EC, 89/336/EEC

Contents

Trademarks **3**
Introduction **4**
Safety **5**
EMC / EMI **6**
Approvals **6**
Certificate of Conformity **7**
About this manual **10**
Unpacking the myograph system **11**

Chapter 1 - System overview **12**
1.1 Myo-Interface front fanel 12
1.2 Myo-Interface rear panel 12
1.3 Auto dual wire myograph unit 13
1.4 Auto dual wire myograph unit with chamber cover 13

Chapter 2 - Setting-up **14**
2.1 The complete myograph 510A system 14
2.2 Setting up step-by-step 14
2.3 The first weight calibration 15

Chapter 3 - The Myo-Interface **16**
3.1 Myo-Interface manual 16
3.2 Menus and submenus 18

Chapter 4 - The Auto dual wire myograph unit **26**
4.1 Adjustment of supports 26
4.2 Calibration of force transducer 28
4.2.1 Principles of weight calibration 28
4.2.2 Weight calibration procedure 29
4.3 Checking or replacing force transducer 32
4.3.1 Simple transducer check 32
4.3.2 Force transducer replacement 32
4.4 Myograph maintenance 34
4.4.1 Myograph chamber pipes 34
4.4.2 Force transducer 34
4.4.3 Linear sides 35
4.4.4 Myograph cleaning 36

Chapter 5 — Getting started **38**
5.1 Dissection protocol for small mesenteric arteries 38
5.2 Mounting protocol for small arteries 41
5.3 Normalization 45
5.4 Standard start 46
5.5 Endothelium Function 47
5.6 In Vitro Experiment 1: Noradrenaline contractile response 48
5.7 In Vitro Experiment 2: Acetylcholine relaxation curve 49

Appendix 1 — Terms of warranty **53**
Appendix 2 — Service check **54**
Appendix 3 — Shipping instructions **55**
Appendix 4 — Myograph accessories and spare parts **56**
A4.1 General myograph equipment 56
A4.2 Myograph 510A system accessories 57
A4.3 Myograph 510A system spare parts 57
Appendix 5 — Fuse replacement **58**
Appendix 6 — Normalization theory **59**
Appendix 7 — Calibration of eyepiece reticule **61**
Appendix 8 — How to read a millimetre micrometer **63**
Appendix 9 - System specifications **64**

About this manual

This manual contains a complete list of procedures describing how to install, maintain and get started using the Auto dual wire myograph system – model 510A – version 3.3

Chapter 1 provides an overview of the construction and basic features of the Myo-Interface and the Auto dual wire myograph unit.

Chapter 2 describes step-by-step how to set-up a complete 510A wire myograph system including accessories.

Chapter 3 is a complete manual to the Myo-Interface. The chapter describes in detail the construction of the menu system and how to use all the features of the Auto dual wire myograph system.

Chapter 4 contains procedures describing general as well as daily maintenance of the myograph unit; e.g. adjustment of supports, weight calibration of the force transducer and cleaning instructions.

Chapter 5 describes how to get started using the wire myograph system. This includes a complete dissection and mounting procedure for small mesenteric arteries, and a few basic pharmacological experiments.

Appendices contain additional information about Normalization theory, ocular calibration, myograph service, shipping instructions, system specifications, equipment lists (accessories and spare parts), and fuse replacement.

Unpacking the myograph system

Please take a few minutes to carefully inspect your new 510A wire myograph system for damage, which may have occurred during handling and shipping. If you suspect any kind of damage, please contact DMT immediately and the matter will be pursued soon as possible. If the packing material appears damaged, please retain it until a possible claim has been settled.

We recommend that you store the packing material for any possible future transport of the Wire myograph system. In case of transport and the original packing material is unavailable, please contact DMT Sales Department for advice and packing instructions.

After unpacking your new 510A wire myograph system, please use the following list to check that the system is complete:

1. **Myo-Interface unit:**
- Myograph connection cable with a temperature probe
- Power cord (The shape of the AC plug varies by country; be sure that the plug has the right shape for your location)

2. **Auto dual wire myograph unit:**
- Calibration kit (including “bridge”, “balance” and 2 gram weight)
- Chamber cover
- Chamber separator

3. **Accessories:**
- 1 roll of 40µm stainless steel wire
- 1 tube of high vacuum grease
- 1 tube of grease for linear slides
- 4 spare screws for mounting of jaws
- 2 Allen keys
- 1 small screwdriver

4. **Manuals**
- User manual for “Auto dual wire myograph system – Model 510A”
- “Procedures for investigation of small vessels using small vessel myograph”, by Professor M. J. Mulvany, Department of Pharmacology, Aarhus University, Denmark
- 1 CD: Dissection and mounting of small vessels in wire myographs

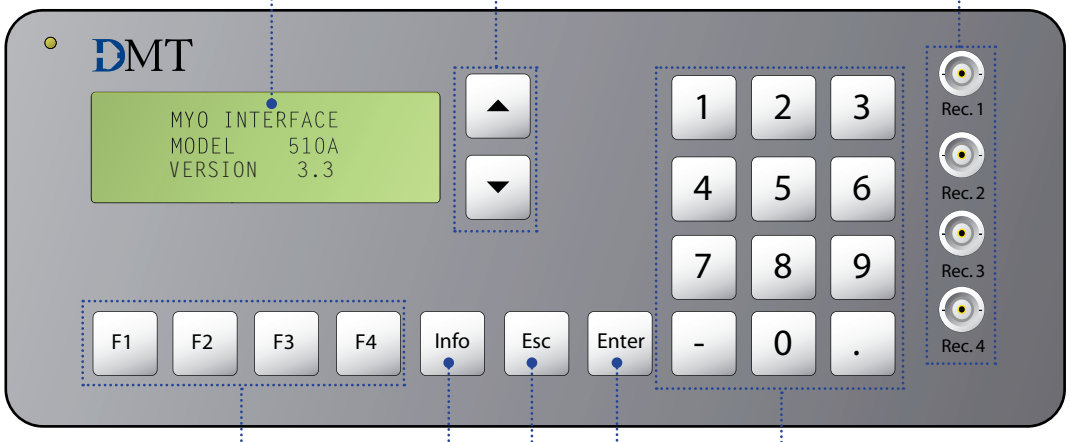
Chapter 1 - System overview

1.1 Myo-Interface front fanel

Up and down arrow keys - Scroll up and down in the menus. If you are at the top of a menu, use ↑ to change to the previous main menu.

Analogue recorder output - Connection to external data acquisition system (optional).

Myo-Interface display



F-Keys - Have different but specific functions dependent of the active main menu.

INFO key - Push this key in any menu to display all specific F-key functions.

Numeric keys - Enter data or setting values

Enter key - Confirm entered numeric values or change between available choices.

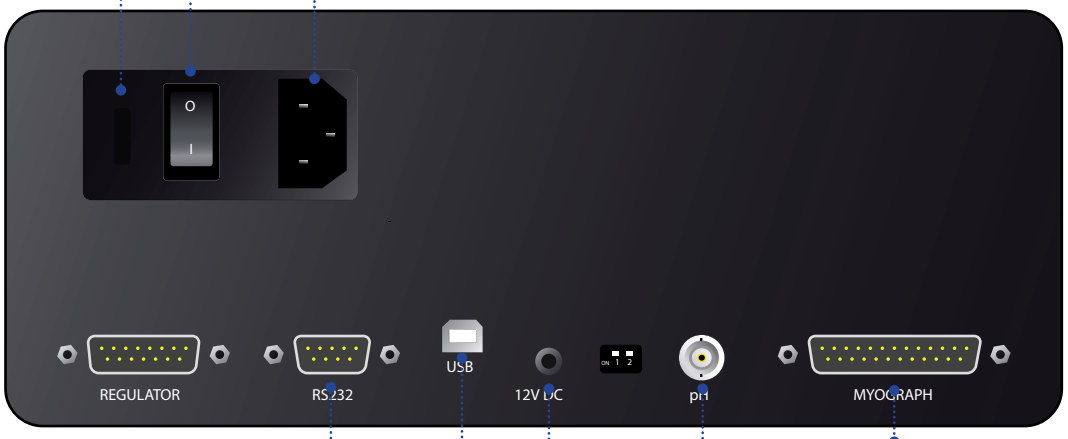
ESC key - Delete incorrectly entered numeric values. In some submenus used to return to main menu.

1.2 Myo-Interface rear panel

115-230 V / 50-60 Hz automatic voltage selector

ON/OFF switch

Power connector



Connection port for myograph unit

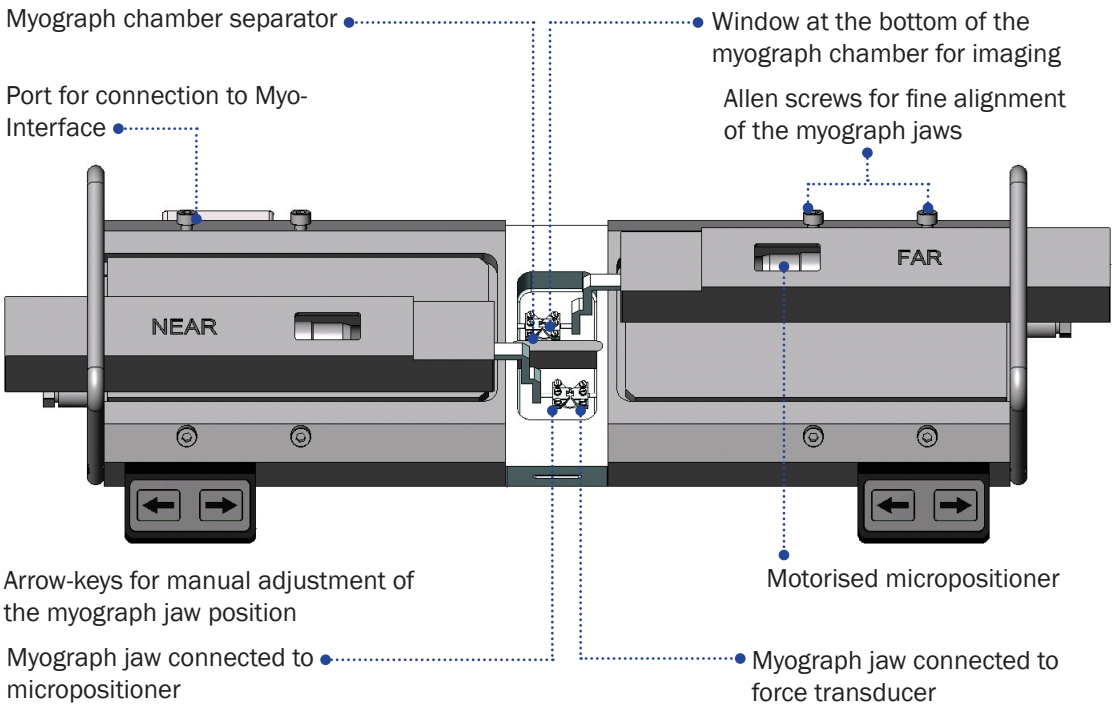
RS 232 port for serial connection to PC

Connection port for pH-Meter (optional)

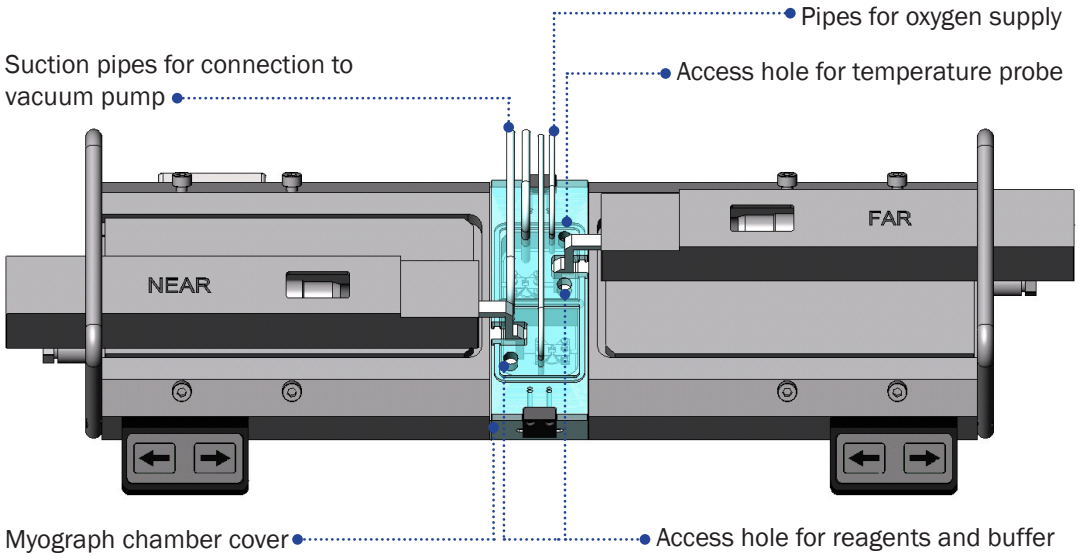
USB port

12V DC Port

1.3 Auto dual wire myograph unit


Myograph chamber separator
Window at the bottom of the myograph chamber for imaging
Port for connection to Myo-Interface
Allen screws for fine alignment of the myograph jaws
NEAR
FAR
Arrow-keys for manual adjustment of the myograph jaw position
Motorised micropositioner
Myograph jaw connected to micropositioner
Myograph jaw connected to force transducer

1.4 Auto dual wire myograph unit with chamber cover



Chapter 2 - Setting-up

2.1 The complete myograph 510A system

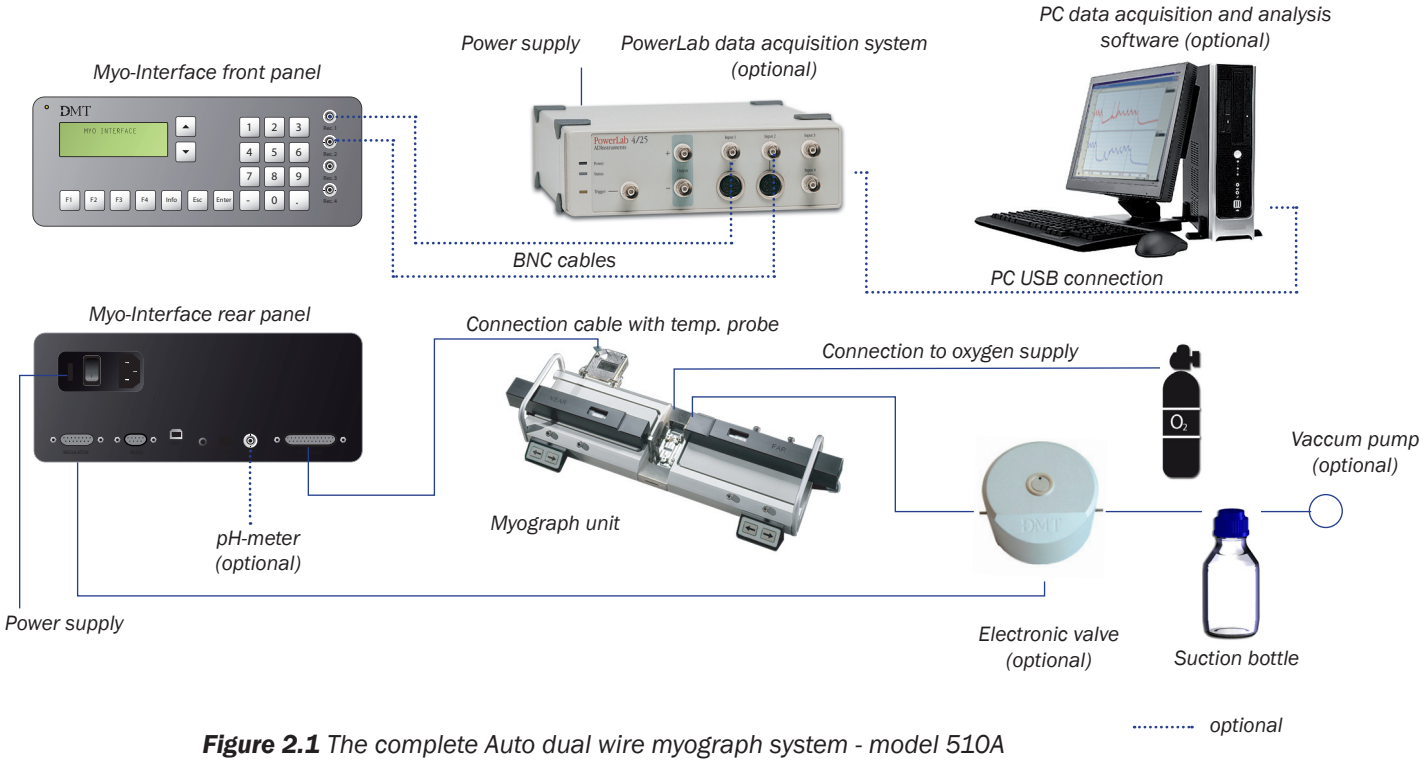


Figure 2.1 *The complete Auto dual wire myograph system - model 510A*

2.2 Setting up step-by-step

The chapter contains a complete step-by-step description of how to set up a complete myograph 510A system as illustrated in fig. 2.1

1. **Myograph unit – Myo-Interface connection:**
   Connect the myograph 510A unit to the Myo-Interface using the grey 25-pin connection cable. The end of the cable with the temperature probe is used with the myograph unit.

2. **Myo-Interface – PC connection:**
   Data acquisition is possible either by connecting the Myo-Interface directly to a PC or through a PowerLab data acquisition and analysis system (optional).

   I. Direct PC connection:
   Connect the Myo-Interface to one of the COM-ports on the PC using a serial cable (not supplied with the system).

II. PowerLab (optional):

Connect the Myo-Interface to the PowerLab unit using BNC cables. Connect Rec 1 on the Myo-Interface to Input 1 on the PowerLab. Rec 2 to Input 2 etc.
Connect the PowerLab unit to one of the USB-ports on the PC using the USB cable delivered with the PowerLab system.

3. **Oxygen supply:**
   Connect the “small” pipes on the myograph chamber cover to an adjustable oxygen supply using small rubber tubes (internal diameter 1.5 mm).

4. **Suction connection:**
   Connect the “large” pipes on the myograph chamber cover to a vacuum pump via a suction bottle and the vacuum valve as illustrated in fig. 2.1. The internal diameter of rubber tubes for connection to the pipes on the chamber cover is 2 mm. An electronic vacuum valve is optional.

5. **pH – electrode (optional):**
   Connect the pH electrode to the pH port on the backside of the Myo-Interface and install the software flash update as described in the purchased procedure. Perform a pH calibration as described in Section 3.1.8.

2.3 The first weight calibration

Prior to the shipment of the 510A System, it has gone through two days of continuous testing including a final weight calibration. However in order to ensure that the myograph is working at highest performance, DMT recommends that a new weight calibration is performed before starting to use the myograph system. The weight calibration procedure is described in detail in Chapter 4.

Chapter 3 - The Myo-Interface

3.1 Myo-Interface manual

When the 510A Myo-Interface is switched on, the display shows that the system is starting up and initializing, after which the start-up message depicted to the right is shown.

MYO-INTERFACE
MODEL 510A
VERSION 3.30
28/02/2008

The display automatically shows the Main menu after several seconds. Otherwise, press **F1** to proceed to the Main menu.
When the start-up message is active, the ▲ and ▼ keys can be used to adjust the display contrast setting.

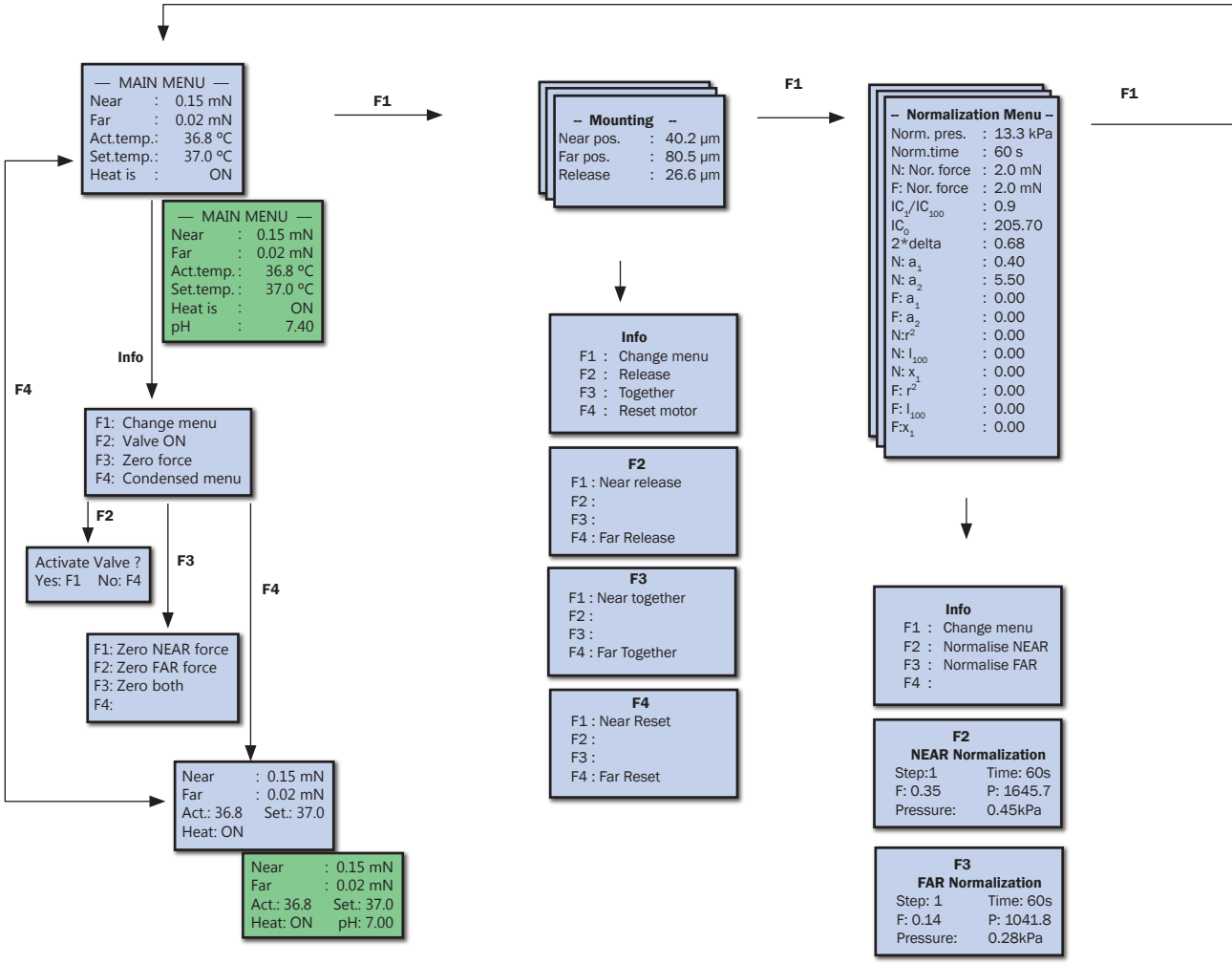
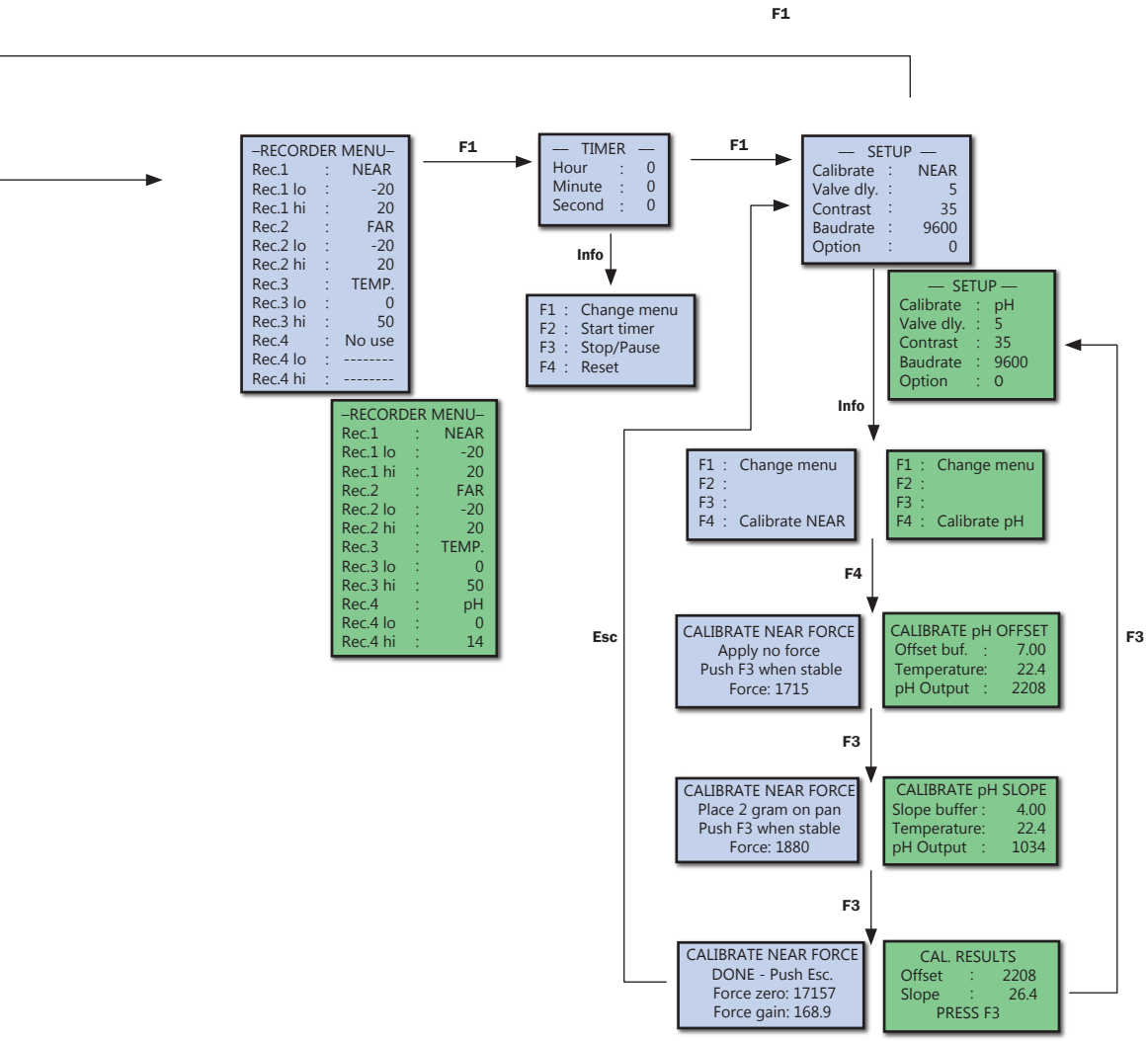


Fig. 3.1: *Menu overview: the green panels indicate the menu appearance/options when the optional pH-meter is installed.*

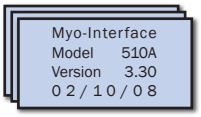

F1
–RECORDER MENU–
Rec.1 : NEAR
Rec.1 lo : -20
Rec.1 hi : 20
Rec.2 : FAR
Rec.2 lo : -20
Rec.2 hi : 20
Rec.3 : TEMP.
Rec.3 lo : 0
Rec.3 hi : 50
Rec.4 : No use
Rec.4 lo : --------
Rec.4 hi : --------
F1
— TIMER —
Hour : 0
Minute : 0
Second : 0
F1
— SETUP —
Calibrate : NEAR
Valve dly. : 5
Contrast : 35
Baudrate : 9600
Option : 0
Info
F1 : Change menu
F2 : Start timer
F3 : Stop/Pause
F4 : Reset
— SETUP —
Calibrate : pH
Valve dly. : 5
Contrast : 35
Baudrate : 9600
Option : 0
Info
–RECORDER MENU–
Rec.1 : NEAR
Rec.1 lo : -20
Rec.1 hi : 20
Rec.2 : FAR
Rec.2 lo : -20
Rec.2 hi : 20
Rec.3 : TEMP.
Rec.3 lo : 0
Rec.3 hi : 50
Rec.4 : pH
Rec.4 lo : 0
Rec.4 hi : 14
F1 : Change menu
F2 :
F3 :
F4 : Calibrate NEAR
F1 : Change menu
F2 :
F3 :
F4 : Calibrate pH
F4
Esc
CALIBRATE NEAR FORCE
Apply no force
Push F3 when stable
Force: 1715
CALIBRATE pH OFFSET
Offset buf. : 7.00
Temperature: 22.4
pH Output : 2208
F3
F3
CALIBRATE NEAR FORCE
Place 2 gram on pan
Push F3 when stable
Force: 1880
CALIBRATE pH SLOPE
Slope buffer : 4.00
Temperature: 22.4
pH Output : 1034
F3
F3
CALIBRATE NEAR FORCE
DONE - Push Esc.
Force zero: 17157
Force gain: 168.9
CAL. RESULTS
Offset : 2208
Slope : 26.4
PRESS F3

3.2 Menus and submenus

General navigation

The following controls are used to display the various menus, choose varying menu options and change values :

F1	Change to the next menu
F2 – F4	Have varying functions depending on the current active menu
Info	Push this key in any menu to display all specific associated F-key functions
Numeric keys	Enter data or setting values
Enter	Confirm entered numeric values or change between available choices
Esc	Delete incorrectly entered numeric values. In some submenus used to return to main menu
▲ – ▼	Scroll up and down through the display, as only four lines are capable of being displayed at a time. Being in the top line of a submenu, use ▲ to change to the previous main menu.



The active line in the menu is indicated by a > symbol.

Turn-on message

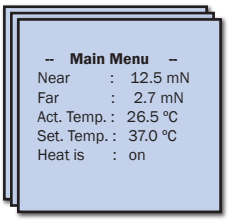
Turning on the 510A Myo-Interface, a message appears as shown to the right. Use the ↑ and ↓ keys to adjust the display contrast setting.

Press **F1** for proceeding to the main menu.

Main menu

The main menu displays:

NEAR:	NEAR myograph force reading in mN
FAR:	FAR myograph force reading in mN
Act. Temp:	Actual temperature probe reading in °C
Set. Temp:	Heating temperature setting in °C
Heat is:	Actual heating status (on/off)



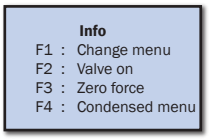
Use the ↑ and ↓ keys to see all values as the display is only capable of showing four lines at the time.

Temperature setting

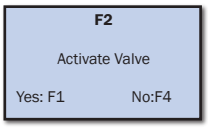
To change the temperature setting, move the Set. Temp line to the top of the display. Use the numeric keys to enter a new temperature setting and press **Enter** to confirm.

Heating control

To turn on heating move the “Heat is” line to the top of the display and use **Enter** to switch between on and off.

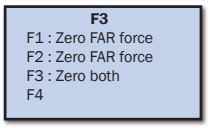


The Main Menu has the following functions:

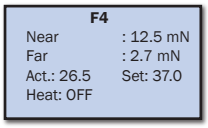


Info: Press the Info key and the display shows the F-key options in the Main menu.

Press **Info** again to return to the Main menu or one of the F-keys to proceed with one of these options.



F1: Press **F1** to proceed to the Recorder menu.



F2: Press **F2** to open the Valve activation Menu. An electronic vacuum valve is available as optional to the standard manual valve. The electronic vacuum valve is connected to the “regulator” port on the backside of the myo graph.

Press **F1** to activate the electronic vacuum valve.
Press **F4** to close the electronic vacuum valve.

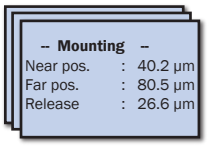
F3: Press **F3** to open the Zero force menu.

Press **F1** to zero NEAR force reading.
Press **F2** to zero FAR force reading.
Press **F3** to zero both NEAR and FAR force readings at the same time.

F4: Press **F4** for the display to show a condensed “Main Menu” showing NEAR/FAR force read ings, actual/set temperature, heat status on/off and pH reading (optional).

Mounting menu

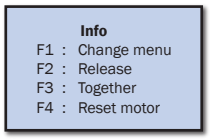
The mounting menu displays:



Near pos.:	Position of the NEAR myograph jaws.
Far pos.:	Position of the FAR myograph jaws. The readings in µm are the actual distance of separation between the jaws.
Release:	The reading in µm shows the release set value. The setting is the apart distance that the jaws will move on to when commanded to do so in the **F2** Release menu as described below.

The position of the jaws may also be adjusted manually by using the ← and → keys on the myograph unit. The speed and behaviour of the motor can be adjusted in the Setup menu by entering the code 1235 in the option line. (see p. 25)

The “Mounting menu” has the following functions:



Info: Press the **Info** key and the display shows the **F**-key options in the Mounting menu.

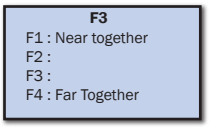
Press Info again to return to the Mounting menu or one of the F-keys to proceed with one of these options.

F1: Press **F1** to proceed to the Normalization menu.

F2: Press **F2** to open the Release menu.

Press **F1** to release NEAR myograph jaws.
Press **F4** to release FAR myograph jaws.

The jaws will then move apart until the distance between the jaws is equal to the release set value.



F3: Press **F3** to open the Together menu.

Press **F1** to move NEAR myograph jaws together.
Press **F4** to move FAR myograph jaws together.

F4: Press **F4** to open the Reset menu.

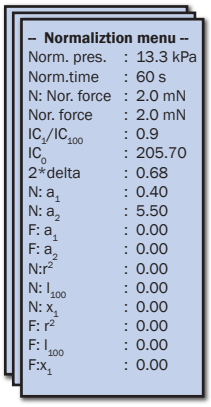
Press **F1** to reset the NEAR myograph motor.
Press **F4** to reset the FAR myograph motor.

DMT recommends that both motors are reset before any experiment is performed.

Normalization menu

The Normalization menu displays:

Norm. pres:	Target transmural pressure, usually 13.3 kPa (100 mmHg)
Norm. time:	Time setting in seconds, defining the duration of each step in the automatic normalization.
N: Nor. force:	NEAR myograph force reading in mN
F: Nor. force:	FAR myograph force reading in mN.
IC_1/IC_{100}:	IC_{100} is the internal circumference corresponding to a certain target transmural pressure, typically 13.3 kPa (100 mmHg). IC_1 is the normalized internal circumference calculated from IC_{100} by multiplying it with a factor less than 1, usually 0.9.
IC_0:	Internal circumference when the wires are just touching. $IC_0 = (2+\pi)\cdot$wire diameter. IC_0 (40 µm wire) = 205.7 µm
2*delta:	Delta is the eyepiece calibration in mm/div. Enter the 2*delta value.
N: a_1:	Microscope eyepiece reading from the far end of the mounted segment.
N: a_2:	Microscope eyepiece reading from the near end of the mounted segment.
F: a_1:	Microscope eyepiece reading from the far end of the mounted
F: a^2:	Microscope eyepiece reading from the near end of the mounted segment.
N: r^2:	Regression coefficient for fit of (X_i, Y_i) to an exponential curve.
N: l_{100}:	Internal diameter of the mounted segment corresponding to a certain target transmural pressure, typically 13.3 kPa (100 mmHg).
N: x_1:	The micropositioner setting required to stretch the mounted segment to its normalized internal circumference.
F: r^2:	Regression coefficient for fit of (X_i, Y_i) to an exponential curve.
F: l_{100}:	Internal diameter of the mounted segment corresponding to a certain target transmural pressure, typically 13.3 kPa (100 mmHg).
F: x_1:	The micropositioner setting required to stretch the mounted segment to its normalized internal circumference.

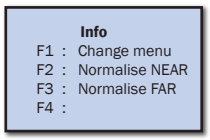


Use the ↑ and ↓ keys to see all values as the display is only capable of showing four lines at the time. To change settings and values in the Normalization menu, move the line to be changed to the top of the display. Use the numeric keys to enter a new setting/value and press Enter to confirm.

The Normalization menu has the following functions:

Info: Press the Info key and the display shows the **F**-key options in the Normalization menu.

Press Info again to return to the Normalization menu or one of the F-keys to proceed with one of these options.

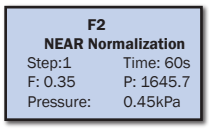


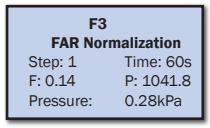
F1: Press **F1** to proceed to the Recorder menu.

F2/F3: After entering all necessary settings and values in the Normalization menu, press:

F2 to start normalization of the segment in the NEAR myograph.
F3 to start Normalization of the segment in the FAR myograph.

Press **ESC** to cancel the normalization.





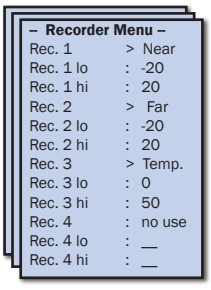
As the Normalization reaches the target transmural pressure (usually 13.3 kPa = 100 mmHg), the Normalization position is calculated and the micropositioner is turned automatically to the correct position. The results of the Normalization are shown afterwards in the Normalization menu.

Recorder menu

The 510A Myo-Interface has four analog output ports at the front panel for connection to a data recording system. Each of these output ports can be programmed to represent either NEAR myograph, FAR myograph, temperature or pH-meter. The default settings are:

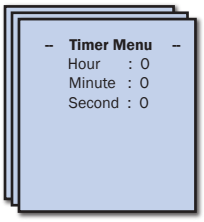
Rec. 1:	NEAR myograph force output
Rec. 2:	FAR myograph force output
Rec. 3:	Temperature output

The full-scale output on the recorder is one volt. In the Recorder menu it is possible to change the value that corresponds to 0 V (lo) and 1 V (hi).

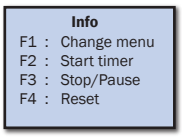

-- Recorder Menu --
Rec. 1 > Near
Rec. 1 lo : -20
Rec. 1 hi : 20
Rec. 2 > Far
Rec. 2 lo : -20
Rec. 2 hi : 20
Rec. 3 > Temp.
Rec. 3 lo : 0
Rec. 3 hi : 50
Rec. 4 : no use
Rec. 4 lo : __
Rec. 4 hi : __

Timer menu

The Timer menu is a feature making it possible to set an alarm based on a count down from a predefined set value of hours, minutes and/or seconds.



To set the count down time, move the line to be programmed (hour, minute or second) to the top of the display. Use the numeric keys to enter the time value and press Enter to confirm.



The Timer menu has the following functions:

- **Info:** Press the Info key and the display shows the F-key options in the Timer menu.

- Press **Info** again to return to the Timer menu or one of the F-keys to proceed with one of these options.

F1: Press **F1** to proceed to the Setup menu.

F2: Press **F2** to start the count down after the time values have been entered in the Timer menu.

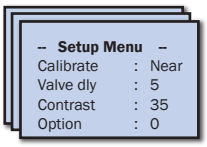
F3: Press **F3** to stop or pause the count down.

F4: Press **F4** to reset entered time values or to turn off the alarm.

Setup menu

The Setup menu displays:

Calibrate:	Choose which sensor to be calibrated. (NEAR/FAR myograph force or pH).
Valve dly:	An electronic vacuum valve is available optional to the standard manual vacuum valve. Valve delay is the time in seconds that the valve stays open when activated.
Contrast:	The actual contrast setting in the display.
Option:	The option line allows access to submenus using special codes.

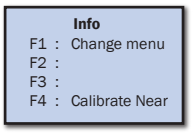


To change any of the settings in the Setup menu, move the line to be changed to the top line of the display:

- Press **Enter** to change between calibration of NEAR myograph force, FAR myograph force or pH.
- Use the numeric keys to change the valve delay or display contrast settings and press **Enter** to confirm.
- **Enter** the specific access code and press Enter to go to the submenu.

The Setup menu has the following functions:

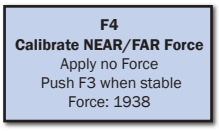
Info: Press the Info key and the display shows the F-key options in the Setup menu.



Press Info again to return to the Setup menu or one of the F-keys to proceed with one of these options.

F1: Press **F1** to proceed to the Main menu.

F4: **Transducer calibration**



Having chosen which one of the myograph force transducers to calibrate (NEAR or FAR), press **F4** to enter the window shown to the right.
Make sure that no force is applied on the force transducer and when the relative reading at the bottom line of the window appears stable, press F3 to proceed.

Carefully place the 2 gram weight on the load pan and when the relative reading at the bottom line of the window appears stable, press **F3** to proceed.

The calibration is now finished. The readings in the two bottom lines are the calibration parameters stored in the internal memory of the Myo-Interface. Press **ESC** to return to the Setup menu.

For further information about the calibration procedure, see chapter 4.

pH calibration (optional)

Having chosen to calibrate the pH settings, press F4 to enter the window shown to the right.
The first line shows the value of the offset buffer (first buffer solution), which is always 7.00.
The second line shows the temperature of the buffer solution. The temperature is an important parameter in the calibration formula and is obtained automatically by placing the myograph temperature probe in the buffer solution.
The third line shows the output from the pH probe as raw data from the A-D converter.

Place the pH-meter electrode and temperature probe in the offset buffer solution and turn on stirring. When the relative pH output in the bottom line is stable, press **F3** to proceed.

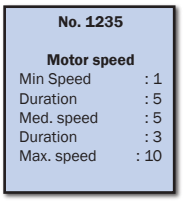
In the first line is now shown the value of the slope buffer (second buffer solution), which is always 4.00.

Place the pH and temperature probes in the slope buffer solution and turn on stirring. When the relative pH output in the bottom line is stable, press **F3** to proceed.

The calibration is now finished. The parameters are stored in the internal memory of the Myo-Interface. Press ESC to return to the Setup menu.

Special motor speed submenu:
To open the special "Motor Speed" submenu, enter the code 1235 in the option line in the Setup menu.

Min. Speed:	Initial speed.
Duration:	The time the motor will run in Min. Speed before it accelerates to Med. Speed.
Med. Speed:	Medium speed.
Duration:	The time the motor will run in Med. Speed before it accelerates to Max. Speed.
Max. Speed:	Maximum speed.



The menu enables adjustment of the motor behaviour when using the arrow-keys on the myograph unit. Each setting can be adjusted between 1 and 10, where 1 is the lowest speed and 10 is the fastest speed.

Press F1 to return to the Setup menu.

Default motor speed settings are shown in the figure to the right.

Chapter 4 - The Auto dual wire myograph unit

Chapter 4 contains a complete explanation of how to adjust, calibrate and maintain the myograph 510A unit to ensure the equipment is always performing to the highest standard.

4.1 Adjustment of supports

A successful mounting of any kind of tubular tissue segment in the wire myograph is to a high extent dependent on perfectly matching supports. The supports are matched prior to the shipment but daily use of the myograph system and greasing of the transducer pinhole will over time create a need for an adjustment of the supports.

Adjustment of the supports is performed using the following step-by-step procedure. Please note that the amount of force on the screws should be very little to avoid breaking the threads. The procedure is illustrated in fig. 4.1.

1) Carefully loosen screw (D_1) on the top of the support connected to the force transducer. Align the horizontal support and carefully tighten the screw again.

2) Loosen screw (D_2) on the top of the support connected to the linear slide. Align the horizontal support matching the force transducer connected support as carefully as possible and gently tighten the screw again.

3) Loosen screw (C) on the linear slide to roughly match the linear slide support to the force transducer support in the horizontal plane as illustrated in fig. 4.1 (1). Tighten the screw before proceeding with step 4).

4) The plate (B), on which the linear slide is mounted is balanced on top of a small stainless steel ball making it possible to finely adjust the linear slide support in all vertical and horizontal planes using the four Allen screws (A). Use the four Allen screws to make the final horizontal (fig. 4.1 (1)) and vertical (fig. 4.1 (2)) adjustments to match the linear slide support to the force transducer support. The correct matching of the supports is illustrated in fig. 4.1 (3) and (4).

Important:
Be careful not only to continuously tighten the Allen screws when doing the final adjustments. Always remember to loosen the Allen screw placed diagonal to the Allen screw being tightened or else there is a high risk of damaging the myograph frame.

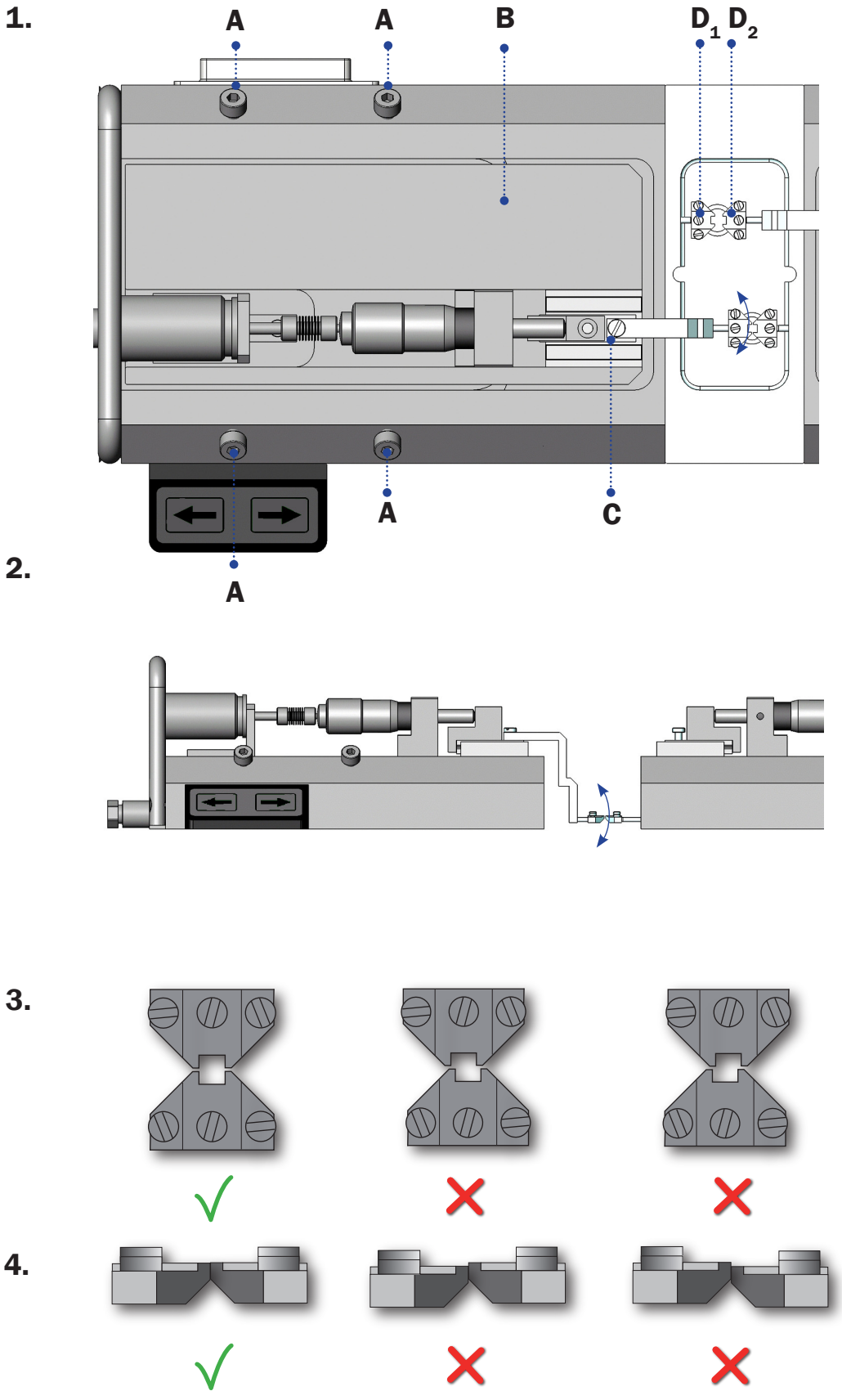


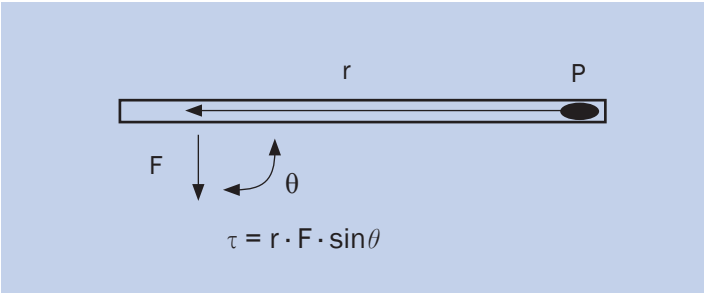
Figure 4.1 *Illustration of how to adjust supports*

4.2 Calibration of force transducer

As a part of the general maintenance of the myograph, DMT recommends that the myograph is weight calibrated at least once every month. DMT also recommends that the myograph is weight calibrated every time the system has been moved or has not been used for a long period of time.

4.2.1 Principles of weight calibration

Weight calibrating the force transducer is based on simple physics: the net torque acting on a balance when applying a certain amount of weight. The magnitude of the torque τ about a point of rotation P is defined by:



where r is the distance from the point of rotation to the point on the object where the force **F** is acting with the angle of θ. Applying the physics to the weight calibration setup is illustrated in fig. 4.2.

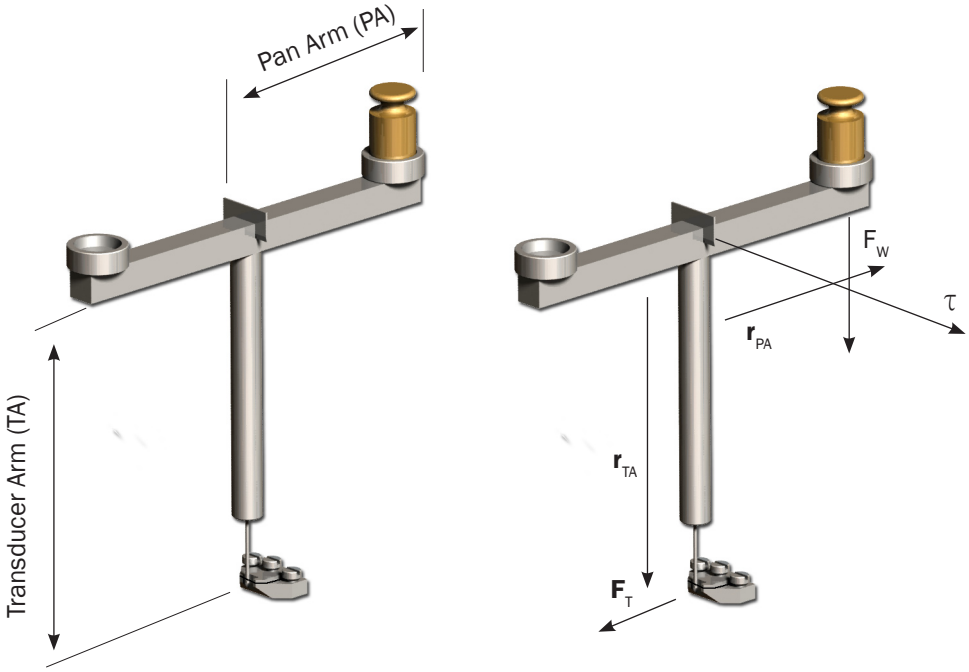


Figure 4.2 *Thereotical principle of the weight calibration*

Applying the weight on the pan arm creates a net torque acting at the "center of gravity" resulting in a force F_T acting on the force transducer. The following two equations describe the forces working in the weight calibration system:

$$1)\ \tau = r_{PA} \cdot F_W \cdot \sin\theta_1 = r_{PA} \cdot (m_{weigt} \cdot g) \cdot \sin\theta_1$$

$$2)\ \tau = r_{TA} \cdot F_T \cdot \sin\theta_2$$

where r_{PA} is the length of the "pan arm". F_W is the force acting on the "pan arm" when applying the weight. F_W is equal acceleration of gravity times the mass of the weight. r_{TA} is the length of the "transducer arm" and F_T is the force acting on the force transducer.

The net torque acting at "center of gravity" is constant for the weight calibration setup, which makes equation 1 and 2 equal, making it possible to calculate the force acting on the force transducer:

$$r_{TA} \cdot F_T \cdot \sin\theta_2 = r_{PA} \cdot (m_{weigt} \cdot g) \cdot \sin\theta_1$$

$$F_T = \frac{r_{PA} \cdot (m_{weigt} \cdot g) \cdot \sin\theta_1}{r_{TA} \cdot \sin\theta_2}$$

As the length of the "pan arm" is 2cm, the length of the "transducer arm" is 4 cm, the weight is 2g, both angles are 90^0 and the acceleration of gravity is 9.81 ms^{-2}, the force acting on the force transducer is:

$$F_T = \frac{2cm \cdot (2\ gram \cdot 9.81\ ms^{-2}) \cdot \sin 90}{4cm \cdot \sin 90}$$

$$F_T = 9.81\ gram \cdot m \cdot s^{-2}$$

As 1N is equal $1kg \cdot m \cdot s^{-2}$, F_W is equal to:

$$F_T = 9.81\ mN$$

4.2.2 Weight calibration procedure

This section contains a complete step-by-step description of how to weight calibrate the force transducers.

1. Fill the myograph chamber with double distilled water and move the jaws apart. Mount a 40 µm stainless steel wire on the jaw connected to the force transducers.

2. Place the calibration bridge, balance and weight randomly on the myograph unit allowing it to be warmed up together with the myograph unit. Turn on the heating in the Main menu on the Myo-Interface.

3. After approximately 20-30 minutes the whole system will have reached the target temperature (normally 37^0C). Place the warmed calibration bridge and balance on the myograph unit as illustrated in fig. 4.3.

4. Make sure that the tip of the “transducer arm” on the balance is placed in the gap between the wire and the jaw as illustrated in fig. 4.4. Carefully move the calibration bridge until the tip of the “transducer arm” is placed freely in the gap, which means it does not touch either the wire or the jaw.

Note: The weight should not be placed on the balance yet.

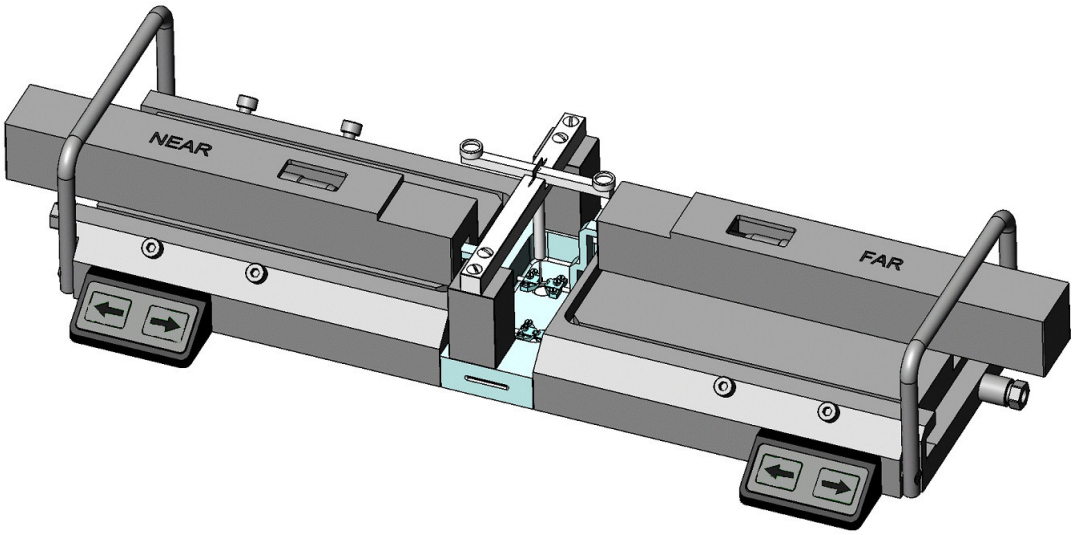


Figure 4.3 *Weight calibration setup*

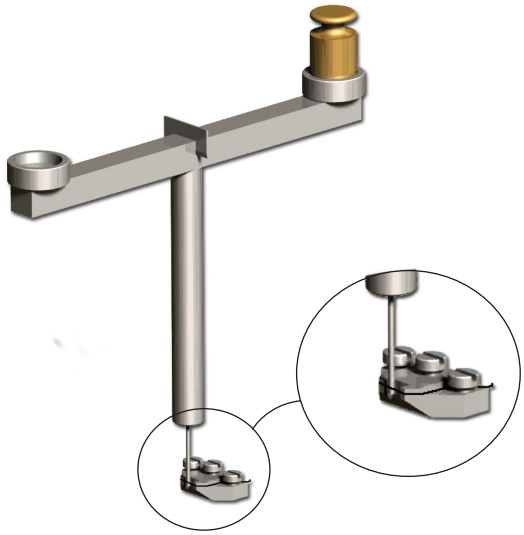


Figure 4.4 *Illustration of how to fit the balance between the wire and the gap in the support.*

5. Go to the Setup menu on the Myo-Interface and choose to calibrate one of the transducers (to change between NEAR and FAR refer to Chapter 3). Press F4 to start calibration.

6. Make sure that absolutely no force is applied on the force transducer by checking that the tip of the “transducer arm” is not touching either the wire or jaw. Also check that the relative force reading in the display is stable. Press F3 to proceed with calibrating.

7. Carefully place the 2 g weight on the pan as illustrated in fig. 4.4. The force applied on the force transducer should mimic the stretch created by the contraction of a mounted ring preparation. Wait until the relative force reading is stable. Press F3 to finish the calibration.

8. Press Esc and go to the Main menu on the Myo-Interface. The force reading on the Myo-Interface is now weight calibrated to an output of 9.81 mN.

Note: If the force reading is unstable or >0.1 mN different from 9.81 mN, repeat the weight calibration. If the instability continues, refer to section 4.3.

9. Carefully remove the weight from the pan and repeat from step 3 to calibrate the other transducer.

9. After calibrating both transducers, carefully remove the weight, balance and calibration bridge and store in the box provided. The myograph system is now ready for use.

4.3 Checking or replacing force transducer

The myograph force transducer is a strain gauge connected in a Wheatstone bridge. The force transducers are placed in separate compartment. The separate compartment provide some mechanical protection for the force transducer but the transducers are still very vulnerable to applied forces exceeding 1 newton (100 g) or fluid running into the transducer compartment due to insufficient greasing of the transducer pinhole.
This section describes how easy it is to check the force transducer and how to replace it if necessary.

4.3.1 Simple transducer check

If the force reading on the Myo-Interface appears unstable then first check that the Myo-Interface and the Myograph unit are properly connected through the 25-pin grey cable.
If the force reading still appears unstable then perform a new weight calibration of the force transducer as described earlier in this Chapter.

During the weight calibration monitor the relative force reading values in the calibration menu on the Myo-Interface:

- If the value is 0 or above 6500 the force transducer is broken and needs to be changed.
- If the reading is between 1–499 or 3001–6250 then contact DMT for further instructions.

If the message “OFF” is displayed in either of the force reading lines in the Main Menu on the Myo-Interface then the force transducer is broken and needs to be replaced.
In case of any other problems related to the force transducer, please contact DMT for further instruction and advice.

4.3.2 Force transducer replacement

In case that the force transducer is broken and needs to be changed, please follow this step-by-step replacement procedure carefully:

1. Disconnect the myograph unit from the Myo-Interface (grey cable).

2. Carefully remove the support connected to the transducer pin by loosening the screw on top of the support as illustrated in fig. 4.1 (1D).

3. Turn the myograph unit up side down and remove the bottom plate by loosening the four screws (A) as illustrated in fig. 4.5 (1)

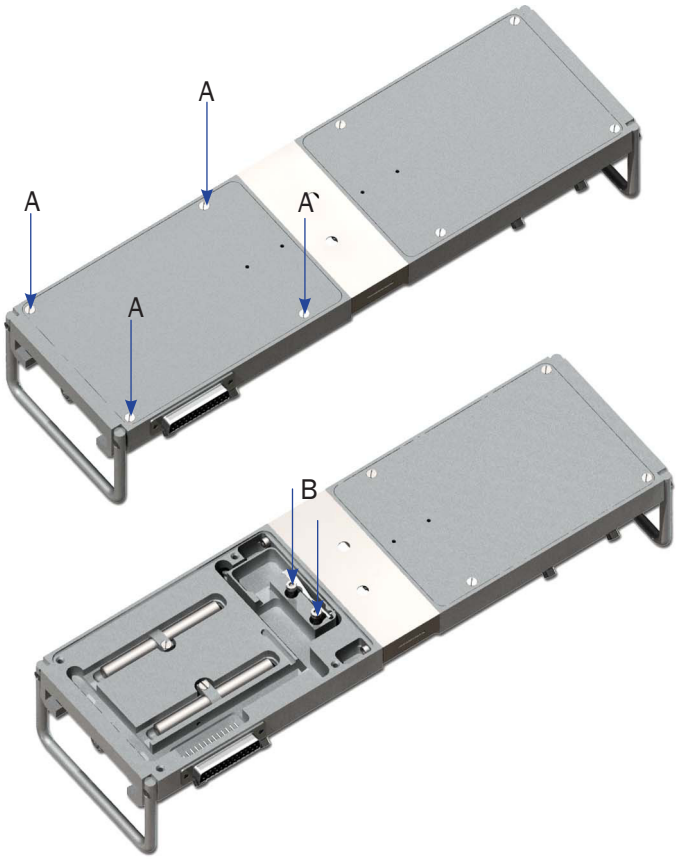


Figure 4.5 *Illustration of how to replace the force transducer*

Important:
Notice how the plug is connected to the force transducer so no mistake is made when connecting the plug to the new force transducer.

4. Carefully disconnect the plug on the force transducer.

5. Loosen and remove the two Allen screws (B) and washers as illustrated in fig. 4.5 and carefully remove the old transducer.

6. Remove any remaining grease from the transducer pin left inside of the transducer compartment of the myograph unit. Clean the hole leading from the transducer compartment to the myograph chamber.

7. Gently place the new force transducer into position. Place the Allen screws and washers in their positions ready to be tightened.

8. Before tightening the Allen screws be sure that the transducer pin is placed directly in the center of the “chamber pinhole” when viewing from the side of the myograph chamber.

9. Tighten the two Allen screws and replace the bottom plate. Tighten the four screws on the bottom plate.

4.4 Myograph maintenance

The Auto dual wire myograph 510A system is a very delicate and sophisticated piece of research equipment, DMT recommend that the following sections are read carefully and that the instructions are followed at all times.

4.4.1 Myograph chamber pipes

To prevent the pipes from being blocked by buffer salt deposits after an experiment, remove the chamber cover from the myograph and turn on the vacuum pump and vacuum valve for about 10 seconds. Wait to turn off the oxygen supply until turning off the vacuum pump. Wipe off any buffer remaining on the outside of the pipes using a piece of paper.

4.4.2 Force transducer

The force transducer is the most delicate and fragile component of the myograph system. Therefore careful handling is necessary.

One of the jaws in each myograph is connected to the transducer pin. The transducer pin enters the myograph chamber through a pinhole in the chamber wall located below the surface level of the buffer as illustrated in fig. 4.6. To prevent the buffer from running into the transducer house the hole is filled with high vacuum grease.As a part of daily maintenance it is very important to inspect the greasing of the transducer hole before starting any experiment. Insufficient greasing causes damage and malfunction of the force transducer.

Important:

- DMT recommends use of the high vacuum grease once a week to seal up the transducer hole by frequently use.
- DMT takes no responsibilities for the use of any other kinds of high vacuum grease than the one to be purchased from DMT.
- DMT takes no responsibilities for any kind of damage applied to the force transducer.

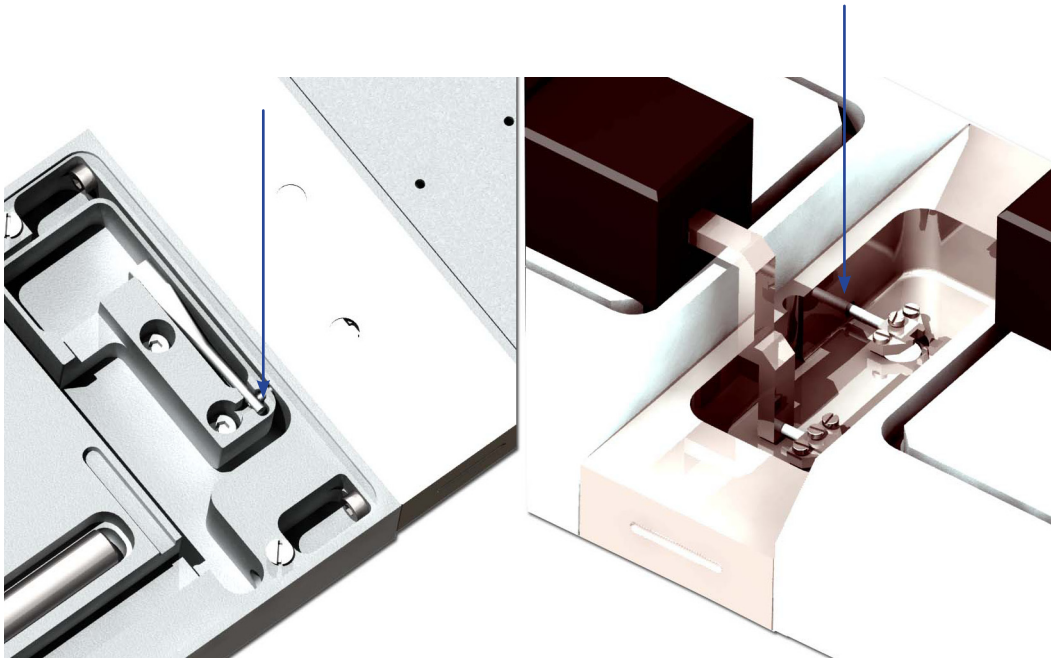


Figure 4.6 *Transducer pin hole to be sealed up with high vacuum grease*

4.4.3 Linear slides

Check the linear slides (under the black covers) for grease at least once a week. In case of insufficient lubrication grease the slides with the original enclosed “Grease for linear slides” at the places marked by the arrows in fig 4.7.

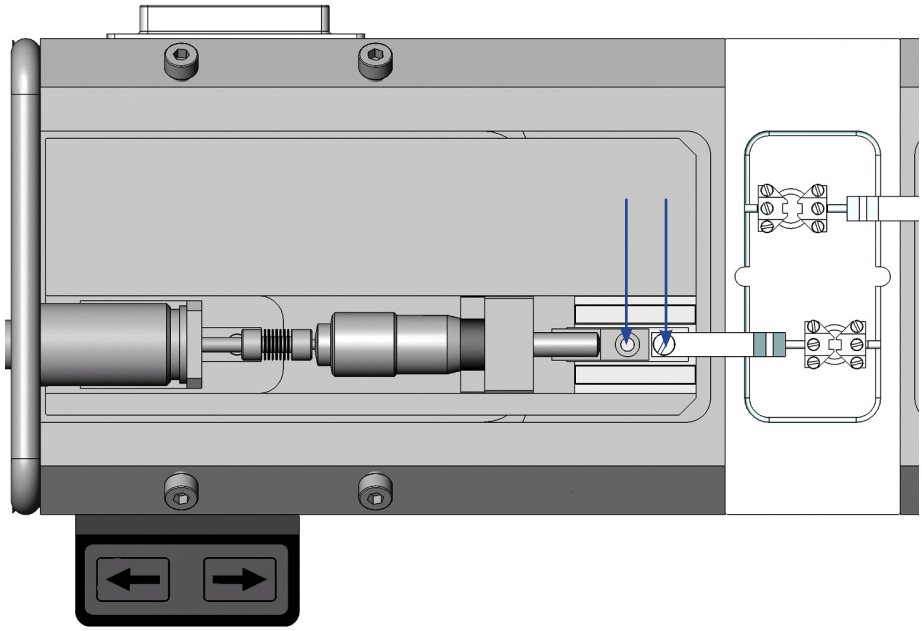


Figure 4.7 *Greasing points on the linear slides.*

4.4.4 Myograph cleaning

DMT strongly recommends that the myograph chamber and surroundings should be cleaned after each experiment.

After a “normal” experiment use the following procedure to clean the myograph chamber and supports:

1. Fill the myograph chamber to the edge with an 8% acetic acid solution and allow it to work for a few minutes to dissolve calcium deposits and other salt build-up. Use a swab stick to mechanically clean all chamber surfaces.

2. Remove the acetic acid and wash the myograph chamber and supports several times with double distilled water.

3. If any kind of hydrophobic reagent have been used, which might be difficult to remove using step 1. and 2. then try incubating the chamber and supports with 96% ethanol or a weak detergent solution (e.g. Treepol).

4. To remove more resistant or toxic chemicals incubate the myograph chamber and supports with 1M HCl for up to 1 hour. In exceptional cases incubate the chamber and supports with an up to 3M HNO_3 solution for about 15 minutes.

5. Wash the myograph chamber and supports several times with double distilled water.

Important Notes:

- Be very careful using step 3. and 4. repeatedly times as strong reagents may cause extreme damage to the myograph unit.

- After cleaning, ALWAYS check that the greasing around the transducer pin is sufficient to keep the buffer solution away from the transducer compartment.

In cases of red or brown discolorations appearing on the chamber sides or on the supports, the following cleaning procedure will work in most cases:

1. Incubate the myograph chamber and supports for 30 minutes with 20µl of a 2mM T-1210 Tetrakis- (2-pyridylmethyl)-ethylenediamine solution dissolved in double distilled water.

2. Use a swab-stick to mechanically clean all the affected surfaces during the last 15 minutes of the incubation period.

3. Wash the myograph chamber and supports several times with double distilled water.

4. Incubate the myograph chamber with 96% ethanol for 10 minutes while continuing the mechanical cleaning with a swab-stick.

5. Remove the ethanol solution and wash a few times with double distilled water. Incubate the myograph chamber and supports with an 8% acetic acid solution for 10 minutes and continue the mechanical cleaning with a swab-stick.

6. Wash the myograph chamber and supports several times with double distilled water.

Important Notes:

- In exceptional cases it may be necessary to demount the supports for cleaning supports and the myograph chamber individually to make sure that all surfaces are clean.

Chapter 5 — Getting started

Chapter 5 contains some practical protocols and procedures describing how to quickly get started using the Single Wire Myograph System. The chapter mostly addresses users who are not yet familiar with the wire myograph technique. However it may contain some hints that will also be useful for the experienced myograph user.

5.1 Dissection protocol for small mesenteric arteries

The Single Wire Myograph technique is versatile in that a large variety of physiological and pharmacological studies of ring preparations from different species are possible. The wire myograph is predominantly used for investigation of small blood vessels and as an example this chapter describes the dissection of rat mesenteric arteries.

1. A laboratory rat is euthanized in accordance to the local national law and regulations. A midline laparotomy is performed to expose the mesenteric bed.

2. Use scissors to remove about 10 cm of intestine together with its feeding vasculature, including part of the superior mesenteric artery. Be careful not to damage the vasculature during this procedure. The proximal end of the intestine section must be about 10 cm from pylorus. Make a cut in the proximal end of the intestine for later identification.

3. Place the excised intestine section in a Petri dish (about 9 cm in diameter) coated with a 5 mm thick layer of Sylgard at the bottom to hold the fixing pins. Immediately fill the Petri dish with cold PSS, which has been well aerated with carbogen. The dissection is performed without further oxygenation of the PSS. The cold temperature ensures the artery is relaxed during the mounting procedure.

4. Pin down the proximal end of the intestine section on the left-hand side of the Petri dish without stretching the vessels. Pin down the remaining of the intestine section in an anti-clockwise direction. In this configuration (proximal end at the left side, distal end at the right side and running anti-clockwise from proximal to distal side) the feeding vasculature is on the far side of the intestine and the veins are usually uppermost.

5. Select the vessel segment to be investigated (fig. 5.1). First time myograph users are recommended to start dissecting and mounting vessel segments from the first or second branch from the superior mesenteric artery (internal diameter ~200-300 µm).

6. Use high quality forceps and ocular dissection scissors to dissect the vessel segment of interest. Start cutting through the mesenteric membrane along both sides of the vessel, about 1-2 mm from the vessel. To avoid accidentally cutting the artery always cut along the length of the vessels and never perpendicular to them (fig. 5.2A-B).

7. Dissect away as much adipose tissue as needed around the vessels to distinguish between the artery and vein. The artery can easily be identified by the following characteristics (fig. 5.3):

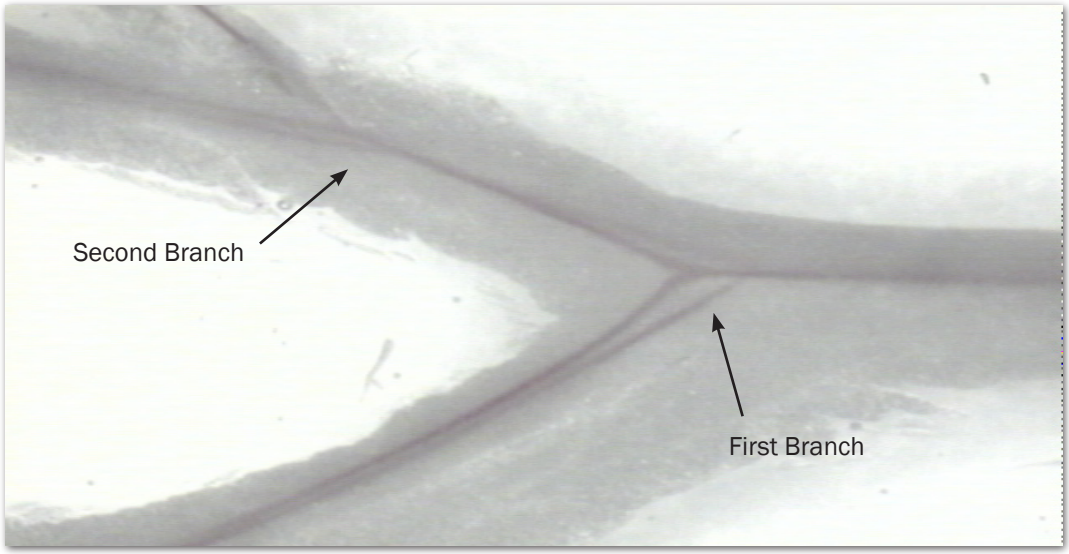


Figure 5.1 *Branching of the mesenteric arteries*

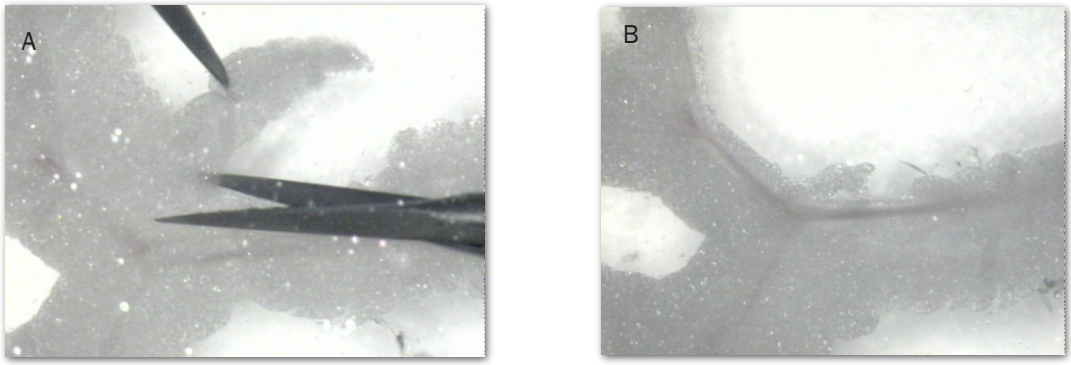


Figure 5.2 *Removal of adipose tissue around the area of interest*

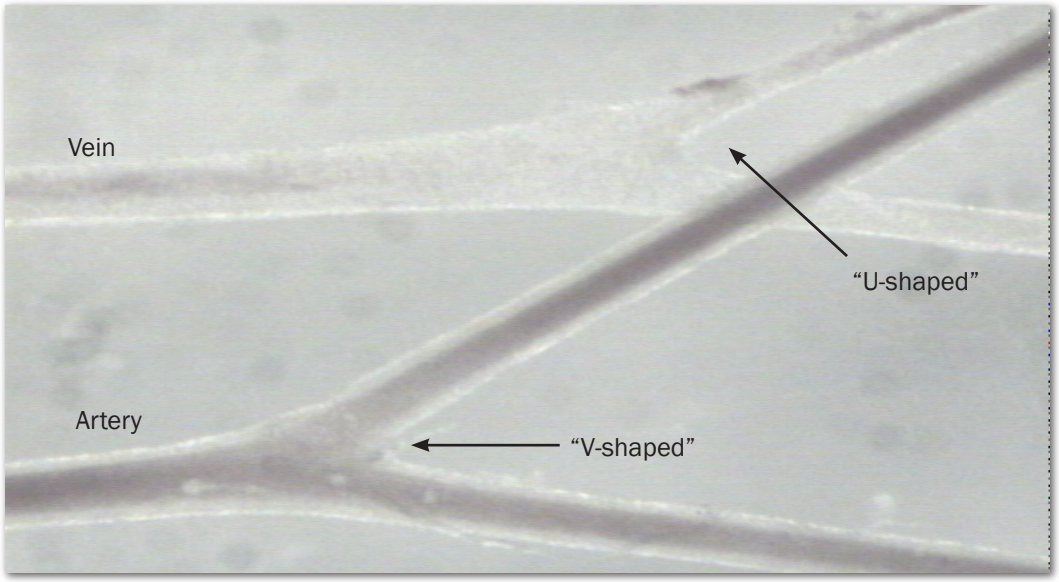


Figure 5.3 *Distinguishing between artery and vein*

- The branch points of arteries are V-shaped whereas those of veins are more U-shaped.
- The arterial wall contains a thick layer of smooth muscle cells compared to the vein wall, which only contains a single or a few layers of smooth muscle cells. The histological difference is clearly visible in the stereomicroscope.
- If you still have difficulty and the vein and artery still contain some blood then try to move the blood forward by very gently squeezing the vessels with a forceps. In the artery the blood will run back quickly whereas in the vein the blood will run back very slowly if it even does so. Note, it is important that you perform this on vessels other than those you will use as this procedure damages the vessels.

8. Dissect away the vein using scissors to cut the adipose and connective tissue between the artery and vein. One method is to cut the vein in one position and afterwards gently to pull the vein away from the artery. In this way a fine membrane of connective tissue becomes visible between the adipose tissue and the artery. Carefully cut the fine membrane to remove the vein and adipose tissue while avoiding any direct contact between the scissor and artery (fig. 5.4 A-B).

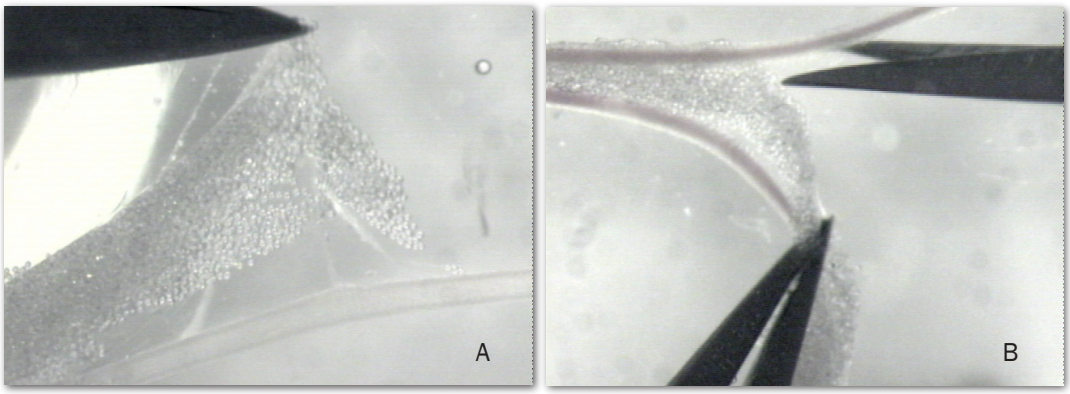


Figure 5.4 *Removal of vein*

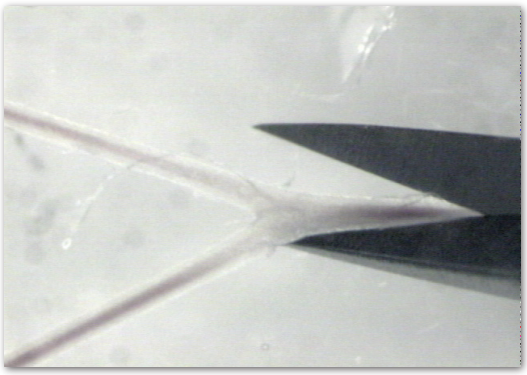
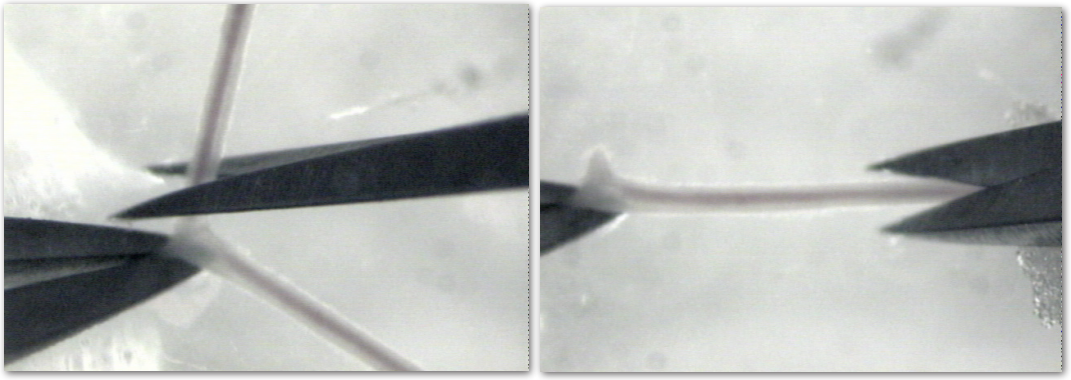


Figure 5.5 A, B and C *Cutting free the artery to be studied*



9. Clean the artery by removing any remaining adipose or connective tissue. Gently pull away adipose or connective tissue to make the connective tissue membrane become visible. Cut the membrane to remove the tissue.

10. Cut the distal end of the artery section to be investigated. Afterwards cut the proximal end while ensuring that the vessel segment has the correct length (fig. 5.5A-C).

5.2 Mounting protocol for small arteries

The procedure involves attaching the mounting wires to jaws which are in turn mounted on the force transducer. This force transducer is capable of measuring with a sensitivity of about 0.01 mN (1 mg), but can be damaged if the applied force exceeds about 1 N (100 g). Therefore care must be taken to avoid pressing the jaws too hard together. A movement of ~20 µm after they have touched is sufficient to hold the wires clamped.

Mounting step one

- Cut lengths of 40 µm wire ~2.2 cm long. Mount one wire on left-hand jaw of myograph as follows.
- Holding wire at far end, place centre of wire between jaws and screw jaws together so that the wire is clamped. (fig. 5.6A) **Note:** do not close the jaws too hard against each other (see above).
- Bend the far end of the wire towards the left, and wrap it around under fixing screw, so the wire is wound clockwise: tightening the screw will then tighten the wire. This procedure should result in the wire being clamped between the jaws and with near end of wire pointing towards operator (fig. 5.6B-C)
- Fill the myograph chamber with PSS (at room temperature).

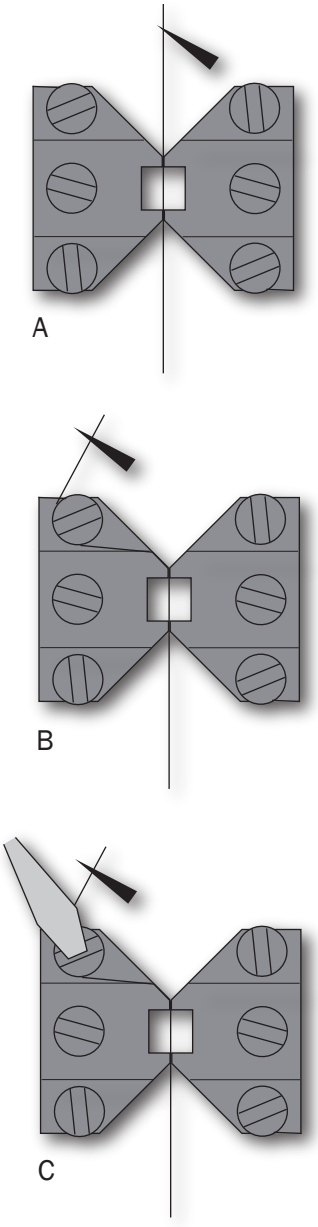


Figure 5.6 A, B and C
Mounting step 1

Mounting step two

- Using forceps to hold the “handle segment, transfer excised vessel from Petri dish to myograph chamber. Hold the vessel as close to the proximal end as possible and try to mount the vessel onto the wire.

If the lumen is shut, try one of the following possibilities:

- Use the wire to gently push the lumen open (blood streaming out is a good sign).
- Hold excised vessel about 3 mm from the cut end with one set of forceps and use the other forceps to squeeze the blood remaining in lumen out through the cut end.
- Pull the proximal end of the excised vessel segment along the wire such that the vessel segment acts as its own feeder to be feed into the wire into the vessel. (fig 5.7A-C). Be careful not to stretch the vessel segment if the end of the wire catches the vessel wall.

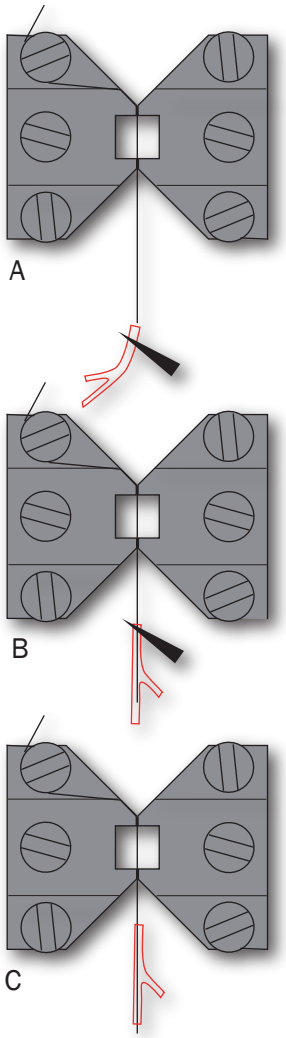


Figure 5.7 A, B and C
Mounting step 2

Mounting step three

- Once the vessel segment is threaded onto the wire, catch the free end of the wire (nearest you) with the forceps and move the jaws apart

- While controlling the movement of the wire with the forceps, use the other forceps to gently pull the vessel segment along the wire until the area of interest is situated in the gap between the jaws. The near end of the vessel segment shall lie about 0.1 mm inside the jaw gap to insure no point of contact (fig. 5.8A)

- Still controlling the free wire end with the forceps, move the jaws together to clamp the wire and in one movement secure the wire under the near fixing screw on the left-hand jaw. Again in a clock-wise direction so that tightening the screw also tightens the wire (fig. 5.8B).

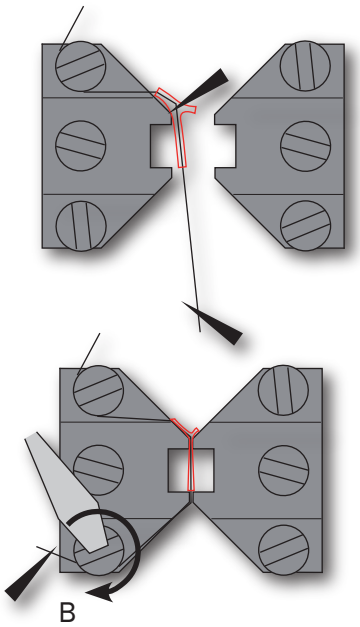


Figure 5.8 A and B
Mounting step 3

Mounting step four

- Using forceps, gently rub the vessel segment on the far side of the jaw to separate any excess vessel segment from the area of interest clamped in the gap between the jaws (fig. 5.9A). Make sure that the vessel segment is separated as close as possible to the jaws. The excessive vessel segment is finally dissected free and removed from the myograph chamber (fig. 5.9B-C).

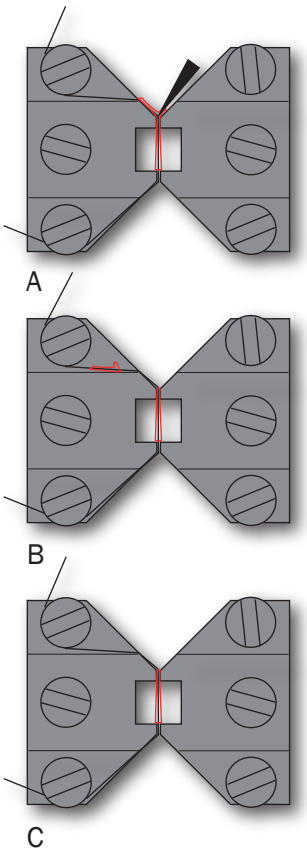


Figure 5.9 A, B and C
Mounting step 4

Mounting step five

- Move the jaws apart (fig. 5.10A). Take a second wire holding it about one third down from the far end using a forceps. Align the wire parallel with the vessel segment such that the wire can be passed into the far end of the lumen. Gently feed the wire through the lumen of the vessel segment in one movement using the first mounted wire as a guide (fig. 5.10B-C). Hold the wire at a point at least 10 mm from the vessel to prevent the vessel being stretched during the manoeuvre. Be careful not to touch the lumen of the vessel with the end of the wire and when pushing the wire end through the near end of the lumen. Once the wire has successfully passed through the lumen of the vessel segment, place the wire in a position, which ensures sufficient length for the wire to be secured both at the near and far fixing screws on the right-hand jaw.

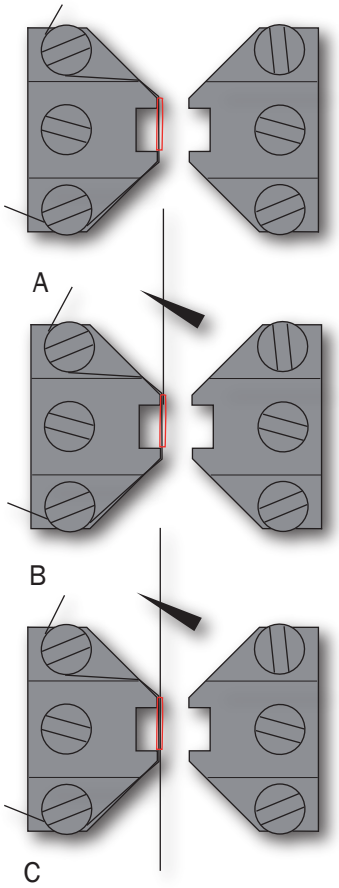


Figure 5.10 A, B and C
Mounting step 5

Mounting step six

- Carefully move the jaws together while ensuring that the second mounted wire lies underneath the first one secured on the left-hand jaw (fig. 5.11A). The procedure clamps the second wire to prevent it from damaging the vessel segment when securing the wire to the right-hand jaw (connected to the transducer). Secure the near end of the wire in a clockwise direction under the far fixing screw on the right-hand jaw (fig. 5.11B).

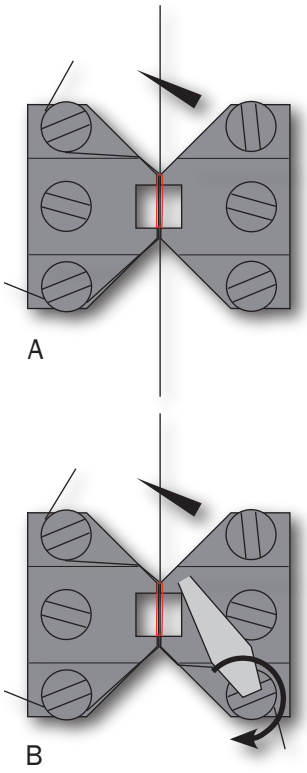


Figure 5.11 A and B
Mounting step 6

Mounting step seven

- Secure the far end of the wire under the near fixing screw on the right-hand jaw. Again the wire is passed clockwise around the screw stretching the wire as the screw is tightened (fig. 5.12A-B). Move the jaws apart to slightly stretch the vessel segment. Make sure that the vessel on the far side of the jaws does not extend beyond the jaws, as even a small extension will affect the Normalization procedure. In case of excess of vessel on the far side of the jaws then move the jaws together again and remove excessive tissue using a forceps as described in mounting step four. A better method for the skilled operator is to move the jaws slightly apart and use scissors to make a small slit in the vessel wall where the vessel is clamped.

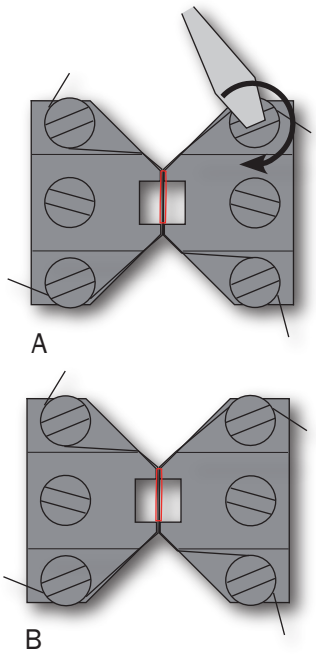


Figure 5.12 A and B
Mounting step 7

5.3 Normalization

The importance of normalizing the preparation is three-fold:

1. Experiments with elastic preparations like vessels can only have meaning if they are performed under conditions where the size is clearly defined.
2. Clearly defined conditions are required in pharmacological experiments as the sensitivity of preparations to agonists and antagonists is dependent on the amount of stretch.
3. The active response of a preparation is dependent on the extent of stretch, which makes it important to set the preparation to an internal circumference giving maximal response.

The aim of the normalization procedure is to stretch the segment to a so-called normalized internal circumference (IC_1): defined as a set fraction of the internal circumference (IC_{100}) that a fully relaxed segment would have at a specified transmural pressure. For small rat arteries the target transmural pressure is typically 100 mmHg = 13.3 kPa.

Principles of the normalization procedure

In practice the normalisation is performed by distending the segment stepwise and measuring sets of micrometer and force readings (fig 5.13, step 1-4). These data are converted into values of internal circumference (µm) and wall tension T (mN/mm) respectively.

Plotting wall tension against internal circumference reveals an exponential curve and by applying the isobar curve corresponding to 100 mmHg, IC_{100} is calculated from the point of intersection using the Laplace relation (fig. 5.14). IC_1 is calculated from IC_{100} by multiplying a factor giving an internal circumference at which the active force production as well as the sensitivity to agonists of the segment is maximal. For rat mesenteric arteries the factor is 0.9 but both this factor as well as the transmural pressure has to be optimised for each particular segment.
The normalized internal diameter is calculated by dividing IC_1 with π.

Appendix 6 , contains a complete description of the mathematical rationale and calculations of the nNormalization procedure.

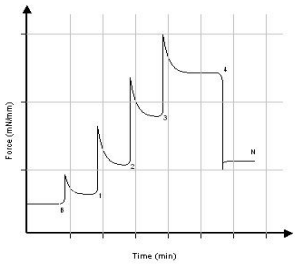


Figure 5.13 *Illustration of the stepwise normalization procedure*

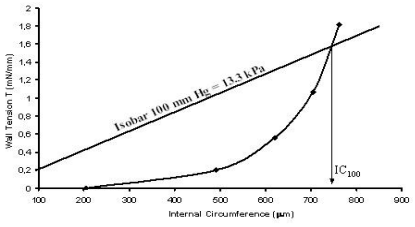


Figure 5.14 *Illustration of the exponential curve fitting and determination of IC_{100}*

5.4 Standard start

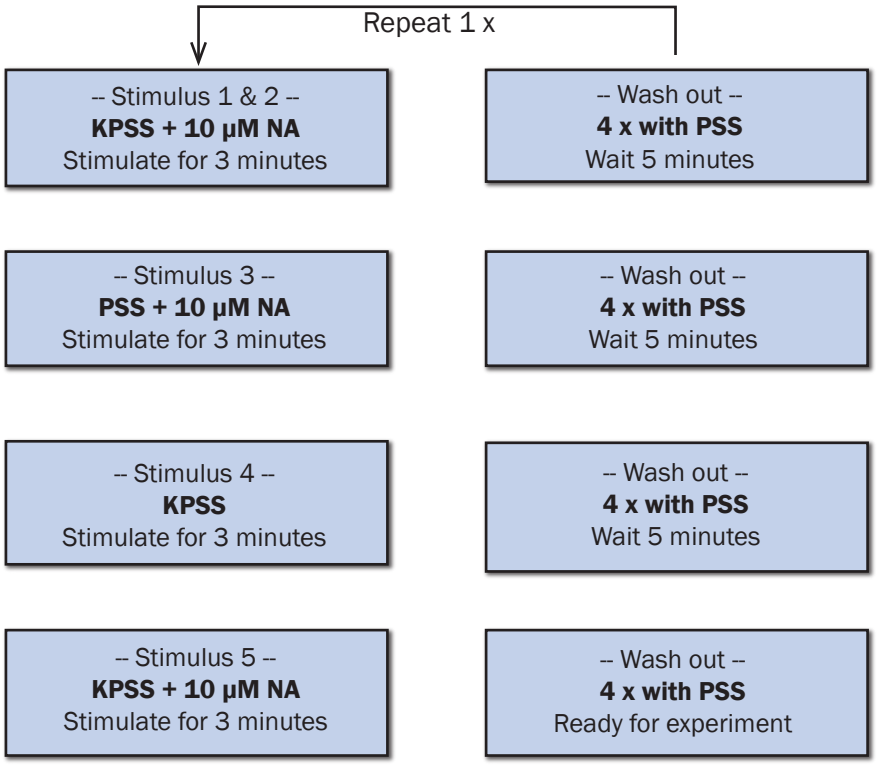
The purpose of performing a standard start is to:

1. Re-activate the mechanical and functional properties of the vessel segment.
2. Check that responses to different types of stimuli are normal in appearance and thereby ensuring that the functionality of the vessel segment has not been damaged during the dissection or mounting procedures.
3. Ensure that the tension development gives an effective active pressure that is above the chosen accepted value (usually 13.3 kPa = 100 mmHg).

The standard start is performed after the vessel segment has been heated, equilibrated and normalised. The present procedure is suitable for rat mesenteric arteries. Another procedure may be needed for other animal species and tissue or vessel types.

Principles of the standard start procedure

The standard start procedure consists of a series of five stimuli and washout periods. The first two stimuli are performed using a mixture of KPSS and 10 µM noradrenaline to give a maximum contractile response. The third stimulus is performed using a mixture of PSS and 10 µM noradrenaline to give a maximum pure agonist mediated (α-adrenoceptor) contraction. The fourth stimulus is performed using KPSS to give a depolarising contractile response (this stimulus also includes a component from neurally released noradrenaline). The final stimulus is performed using a mixture of PSS and 10 µM noradrenaline. All solutions are preheated to 37°C and aerated with a mixture of 95% O_2 and 5% CO_2 before use. Instructions for making the necessary solutions are described at the end of this chapter.



5.5 Endothelium function

The reasons for checking endothelium function may include:

1. To check whether the relaxing function of the endothelium is intact. The procedure is performed to make sure that the endothelium is not damaged during the dissection or mounting procedure.
2. If an experiment requires removal of the endothelium this procedure is useful to check whether the endothelial cells were successfully removed.

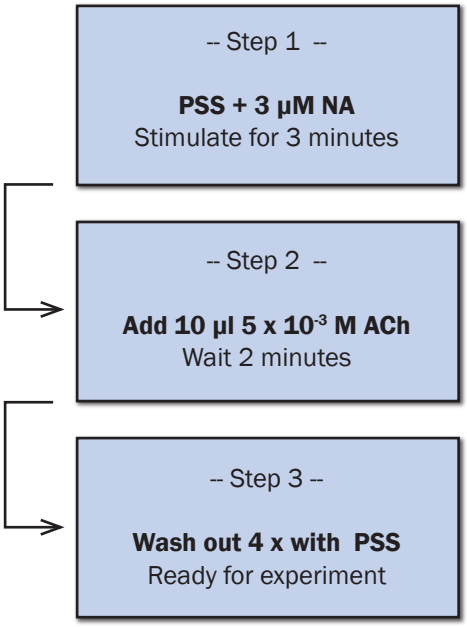
The procedure can be performed after the vessel segment has been heated, equilibrated and normalised. Preferably the procedure should be done after performing a "standard start" to make sure that the vessel segment is viable.

The present procedure is for use with rat mesenteric arteries. Another procedure may be needed for other animal species and tissue or vessel types.

Principles of checking endothelium function

Stimulating a vessel segment with acetylcholine causes a release of nitric oxide (NO, also known as EDRF) from the endothelium cells and subsequent relaxation of the vascular smoth muscle cells. If the endothelium is undamaged by the dissection and mounting procedures, then a substantial relaxation will occur. With complete removal or damaged endothelium, a partial relaxation or no relaxation to acetylcholine is observed.

It is important to note that the amount of NO or EDRF in a vessel is often dependent upon its size. In certain vessels, endothelium-derived hyperpolarising factor (EDHF) can contribute more or less than EDRF, and in other vessels the same stimulation with ACh can promote release of endothelium-derived contracting factor (EDCF). Therefore, it is important to check the existing literature in order to determine the expected response in your particular vessel with the given concentration of agonist.


-- Step 1 --
PSS + 3 µM NA
Stimulate for 3 minutes
-- Step 2 --
Add 10 µl 5 x 10-3 M ACh
Wait 2 minutes
-- Step 3 --
Wash out 4 x with PSS
Ready for experiment

5.6 In Vitro Experiment 1: Noradrenaline contractile response

The purpose of the present protocol is to determine the sensitivity of rat mesenteric small arteries to the vasoconstrictor noradrenaline/norepinephrine with a cumulative concentration-response curve.

Background

Noradrenaline (norepinephrine) causes contraction of mesenteric small arteries through activation of α-adrenoceptors whereas noradrenaline activation of β-adrenoceptors causes vasodilatation. As the purpose is to determine the contraction sensitivity to noradrenaline, the vasodilatory effect of noradrenaline is eliminated throughout the experiment by the constant presence of the β-adrenoceptor antagonist, propranolol.

Rat mesenteric arteries are densely innervated by sympathetic nerves, which have a highly efficient reuptake mechanism that removes noradrenaline from the neuromuscular junction. The reuptake mechanism will create a concentration gradient between the solution around the vessel segment and the receptors on the smooth muscle. To correctly determine the sensitivity to noradrenaline it is necessary to eliminate this concentration gradient by performing the experiment in the presence of cocaine to block the noradrenaline reuptake.

To determine the sensitivity to noradrenaline the vessel segment is exposed to increasing concentrations of noradrenaline. Each concentration is applied until a steady response has been reached and then the next concentration is applied. When the vessel segment is fully contracted or does not response more upon increasing the noradrenaline concentration, the experiment is ended.

Protocol

Prepare the following stock solutions:

- Noradrenaline: 10^{-4}, 10^{-3}, 10^{-2} M
- Propranolol: 10^{-3} M
- Cocaine: 10^{-3} M

1. Mount and normalise the vessels as described in Chapter 5.
2. Perform a standard start as described at start of this chapter.
3. Incubate the vessel segment in 1 µM propranolol (add 5 µL of 10^{-3} M to 5 mL PSS in chamber) and 3 µM cocaine (add 15 µL of 10^{-3} M to 5 mL PSS in chamber) for at least 10 minutes.
4. Add increasing concentrations of noradrenaline into the bath (use Table 6.1 as a guideline). Wait for a stable contractile response or a standard time such as 2 minutes between each application.

[NA] in chamber (µM)*	Volume of stock solution to add to chamber
0.1	5 µL of 10^{-4} M
0.3	1 µL of 10^{-3} M
0.5	1 µL of 10^{-3} M
1	2.5 µL of 10^{-3} M
1.3	1.5 µL of 10^{-3} M
1.5	1 µL of 10^{-3} M
3	7.5 µL of 10^{-3} M
5	1 µL of 10^{-2} M
10	2.5 µL of 10^{-2} M
**In calculating the [NA] in the myograph chamber, the applied volume of noradrenaline is ignored.*	

5.7 In Vitro Experiment 2: Acetylcholine relaxation curve

The purpose of the present protocol is to determine the sensitivity of the endothelium dependent vasodilator acetylcholine in noradrenaline pre-contracted rat mesenteric small arteries.

Background

Acetylcholine causes relaxation of rat mesenteric small arteries by activating of muscarinic M3 receptors at the endothelial cell layer leading to release of endothelium-derived relaxing factors.

Rat mesenteric arteries do not show spontaneous tone in the wire myograph, which is why it is necessary to first induce a contraction to be able to observe the relaxation to acetylcholine. In this protocol the contraction is induced by noradrenaline. The required concentration of noradrenaline needs to be optimised since a too low concentration makes it impossible to evaluate the relaxation. On the other hand it may be difficult to relax super maximally contracted arteries, which may lead to an underestimation of the sensitivity to acetylcholine. Therefore it is recommended to apply a concentration of noradrenaline inducing 60-70% of maximal contraction response. In practice this concentration is found by performing a noradrenaline concentration-response curve as described in the previous section.

The vessel segment is exposed to the noradrenaline concentration and when the response has stabilised, increasing concentrations of acetylcholine are added to relax the vessel. Each concentration is applied until a steady response has been reached and then the next concentration is applied. When the vessel segment is either fully relaxed or does not relax more upon increasing the acetylcholine concentration, the experiment is ended.

Protocol

Prepare the following stock solutions:

- Acetylcholine: 10^{-4}, 10^{-3}, 10^{-2} M
- Noradrenaline: 10^{-2} M

1. Mount and normalise the vessels as described in Chapter 5.
2. Perform a standard start and check the vessel segment for endothelium function, as described at the start of this chapter.
3. Add noradrenaline to obtain a response around 60% of maximum (determined from the previous noradrenaline concentration-response curve). When the contractile response is stable, add increasing concentrations of acetylcholine to the chamber, using Table 6.2 as a guideline. Wait for a stable contractile response or a standard time such as two minutes between each application.

[ACh] in chamber (µM)*	Volume of stock solution to add to chamber
0.1	5 µL of 10^{-4} M
0.3	1 µL of 10^{-3} M
0.5	1 µL of 10^{-3} M
1	2.5 µL of 10^{-3} M
1.3	1.5 µL of 10^{-3} M
1.5	1 µL of 10^{-3} M
3	7.5 µL of 10^{-3} M
5	1 µL of 10^{-2} M
10	2.5 µL of 10^{-2} M
**In calculating the [ACh] in the myograph chamber, the applied volume of ACh is ignored.*	

5.8 Buffer Recipes

Physiological Saline Solution (PSS)

To make 1 L of PSS:

Solution 1

Chemical	**MW (g/mol)**	**Conc. (mmol/L)**	**Conc. (g/L)**
NaCl	58.44	118.99	6.95
KCl	74.56	4.69	0.35
$MgSO_4$ - $7H_2O$	246.48	1.17	0.29
KH_2PO_4	136.09	1.18	0.16

Solution 2

Chemical	**MW (g/mol)**	**Conc. (mmol/L)**	**Conc. (g/L)**
$CaCl_2$ - $2H_2O$	147.02	2.50	0.37

Solution 3

Chemical	**MW (g/mol)**	**Conc. (mmol/L)**	**Conc. (g/L**
$NaHCO_3$	84.01	25.00	2.10
EDTA	372.24	0.03	0.01
Glucose	198.77	5.50	1.09

1. Dissolve the chemicals in approximately 100 mL double distilled H_2O as three individual solutions as described in the table above. Gently heat solution 3 to dissolve the EDTA.
2. Solution 1 is added to a graduated bottle and the bottle is filled with double distilled H_2O to a final volume of 500 mL.
3. Solution 3 is added to the graduated bottle, which afterwards is filled with additional double distilled H_2O to a final volume of about 850 mL.
4. Aerate the solution with carbogen (95% O_2 + 5% CO_2) for about 20 minutes.
5. Solution 2 is added and the graduated bottle is filled with additional double distilled H_2O to reach the final volume of 1000 mL. Continue the carbogen bubbling until the pH of the buffer solution reaches 7.4.

25x Concentrated PSS

To make 1 L concentrated PSS:

Solution 1

Chemical	**MW (g/mol)**	**Conc. (mmol/L)**	**Conc. (g/L)**
NaCl	58.44	118.99	173.85
KCl	74.56	4.69	8.75
$CaCl_2$ - $2H_2O$	147.02	2.50	9.20

Solution 2

Chemical	**MW (g/mol)**	**Conc. (mmol/L)**	**Conc. (g/L)**
$MgSO_4$ - $7H_2O$	246.48	1.17	7.23
KH2PO_4	136.09	1.18	4.02

Solution 3

Chemical	**MW (g/mol)**	**Conc. (mmol/L)**	**Conc. (g/L)**
EDTA	372.24	0.03	0.25

1. Dissolve the chemicals for solution 1 in about 800 mL double distilled H_2O in a 1000 mL graduated bottle. Dissolve the chemicals for solutions 2 and 3 in 75 mL double distilled H_2O in individually cylinders. Gently heat solution 3 to dissolve the EDTA.
2. Solution 2 and 3 is added to solution 1 and the graduated bottle is filled with additional double distilled H_2O to reach a final volume of 1000 mL.

Before use:

3. Dilute the 25 x PSS stock solution 1:25 with double distilled H_2O.
4. Add
   1.091 g/L Glucose
   2.100 g/L $NaHCO_3$
5. Aerate the solution with carbogen (95% O_2 + 5% CO_2) for at least 20 minutes. If necessary wait further for the pH of the buffer to reach pH 7.4.

High-Potassium physiological saline solution (KPSS)

To make 1 L of KPSS:

Use the recipe for regular PSS but replace the desired concentration of NaCl with KCl. For example, to make 60 mM KPSS;

Solution 1

Chemical	**MW (g/mol)**	**Conc. (mmol/L)**	**Conc. (g/L)**
NaCl	58.44	64.86	3.79
KCl	74.56	58.82	4.39
$MgSO_4$ - $7H_2O$	246.48	1.17	0.29
KH_2PO_4	136.09	1.18	0.16

Calcium-free physiological saline solution (Ca-free PSS)

To make 1 L of Ca-free PSS:

Use the recipe for regular PSS but omit the $CaCl_2$ and add EGTA (to buffer the residual Ca^{2+} in solution). The concentration of EGTA in the PSS should be 1-5 mM to ensure sufficient buffering.

Appendix 1 — Terms of warranty

Warranty
DMT A/S warrants to the original user that myograph systems manufactured by DMT A/S will be free from defects in materials and workmanship for a period of three years after the date of delivery. DMT A/S will repair or replace any defective part, subject to the conditions, limitations and exclusions.

Exclusions
Force and pressure transducers, separately or part of myograph systems manufactured by DMT A/S, are disclaimed from any warranty.

Limitations
This warranty shall not apply to equipment subjected to accidental damage, improper use, alteration, or deterioration.

Warranty on third-party products will be as determined by their respective manufacturer.

DMT A/S shall not be liable for consequential, incidental, special, or other direct or indirect damages resulting from economic loss or property damage sustained by you or any end user from the use of the products sold or services rendered hereunder.

Warranty Returns
A “Return Material Authorisation” (RMA) number is required for all returns. This number should be clearly indicated on all returned myograph systems.

Products damaged due to improper or inadequate packaging when returned for RMA purposes are not granted warranty coverage.

Appendix 2 — Service check

A myograph working at optimal performance is extremely important for success when studying small blood vessels or other small tubular tissues. To make sure that our customers always are dealing with first class myographs, DMT offers a Myograph Service Check at a very favourable price.

The Myograph Service Check includes a complete separation for inspection of all mechanical and electronic parts. The myograph is then reassembled, adjusted and finally all electronic and mechanical parts are tested.

Please note that the service does not include replacement of transducers or any other needed spare parts. Please contact DMT for information about prices.

Appendix 3 — Shipping instructions

If the myograph system needs to be sending back for service or repair, please read the following shipping instructions very carefully.

Before you start packing the myograph system, please remember that you are dealing with very delicate equipment and therefore care must be taken. DMT recommends that each part of the myograph system be wrapped individually (i.e. with bubble wrap) and placed together in a large box (preferable the box you once received the myograph system in). Place the wrapped items in the middle of the box and fill out the surroundings with chips of expanded polystyrene.

Important:
Before closing the box, make sure that no enclosed items can be shaken around as transport by road or air from time to time can be quite roughly.

Address the box to:
DMT A/S
Skejbyparken 152
DK-8200 Aarhus N
Denmark

Make sure that all four sides of the box are marked “fragile” or similar. Make an indication on the top of the box that it contains goods returned for repair/service.

Customers outside the EC must further enclose a pro forma invoice stating that the box contains goods being returned for repair or service.

If arranging transportation through a courier, please keep in mind the high value of the myograph system and that a standard insurance provided by the courier in most cases is insufficient to cover damage or loss of the myograph system. In most cases an additional insurance coverage is needed.

Appendix 4 — Myograph accessories and spare parts

This section contains a complete register of equipment needed to set-up a Single Wire Myograph System. In addition the chapter contains a list of special myograph 310A accessories and spare parts.

Besides the main focus on development and manufacturing, DMT has specialised in offering our costumers first class laboratory equipment needed for a Single Wire Myograph setup at very competitive prices. Please contact DMT Sales Department for further product information and prices.

A4.1 General myograph equipment

This sedtion contains a complete checklist of laboratory equipment needed when setting up a Single Wire Myograph system:

- **Dissection stereo microscope**
  (including ocular micrometer and stage micrometer)
  DMT recommends the Zeiss Stemi 2000 Stereo Microscope

- **Mounting forceps** DMT Item # DF-3000
  DMT recommends Dumont Medical No 5 tip (0.10 mm x 0.06 mm).

- **Dissection scissors** DMT Item # DS-1000
  DMT recommends Geuder G-19745 8 cm straight trabeculum.

- **Pipettes**
  DMT recommends CappAeroμ pipettes.

- **Light source**
  DMT recommends Schott Cold Light Source, either Model KL 200 or Model KL 1500 LCD.

- **Water bath including heater**
  DMT recommends Julabo 5 l. open bath circulator with plexiglass bath tank for internal temperature applications.

- **Glass bottle 2 L**

- **Vacuum pump**
  DMT recommends a membrane vacuum pump having a volume of at least 6 litres per minute.

- **Dissection petri dish** DMT Item # PD-2000
  DMT recommends a ~9 cm glass Petri dish coated with a 5 mm Sylgard polymer layer

A4.2 Myograph 510A system accessories

This section contains a list of special accessories available for the Auto dual wire myograph system – Model 510A:

- **ADI PowerLab data acquisition system**
Including Chart data acquisition and analysis software.

- **DMT Normalization module** DMT Item # MLS065
Chart add-on software.

- **pH – Meter**
Including pH electrode and pH-meter.

- **Plastic mounting jaws** DMT Item # PJ-345610
With built-in platinum electrodes for electrophysiological experiments.

- **25 µm Tungsten wire** DMT Item # TW-345610
For mounting of ring preparations with internal diameter down to 60 µm.

- **Electronic vacuum valve** DMT Item # VV-9937

- **Standard PC system** DMT Item # 80150

- **DMT CS-200 (4 Channel) Current stimulator** DMT Item # CS-200
Combined pulse and train generator.

A4.3 Myograph 510A system spare parts

This section contains a complete list of standard spare parts available for the myograph 510A. For parts not listed in this Chapter or for special parts, which may need to be custom made, please contact DMT for further information.

- **Force transducer** DMT Item # FT-61780

- **Roll of 40 µm stainless steel wire** DMT Item # SSW-123456

- **High vacuum grease and grease for linear slides** DMT Item # HVG-1000

- **Stainless steel jaws** DMT Item # SSJ-345610

- **Mounting support pins** DMT Item # MO-200

- **Calibration kit** DMT Item # CK-6127800

- **Chamber cover** DMT Item # CC-61700

- **40 mm funnels** DMT Item # F-2000

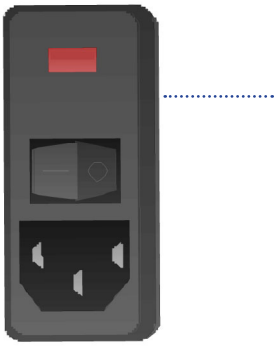
Appendix 5 — Fuse replacement

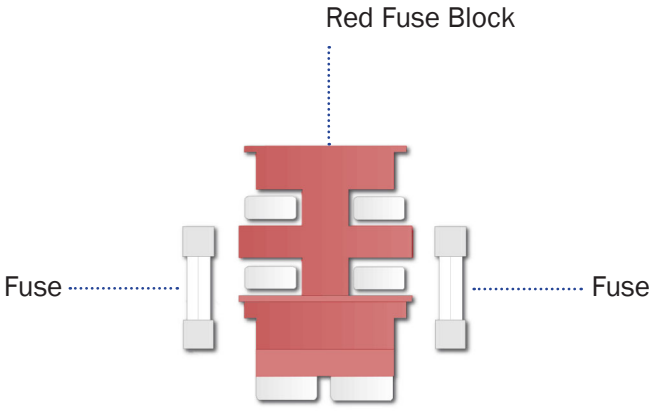
The main fuse of the myograph system is placed inside the power inlet on the Myo-Interface. If the fuse blows it is easily changed using the following procedure.

When a fuse blows and needs to be changed, it is imperative that the replacement fuse is equal to the one blown.
The 310A system uses: T1.6A / 250 V, 6.3 x 32 mm
DMT recommends that both fuses in the fuse block are changed at the same time, as it can be difficult to determine which fuse is blown.

To replace the fuses:

1. Use a small screwdriver to open the voltage selector block.
2. Remove the red fuse block.
3. Remove the existing fuses.
4. Insert the new fuses.
5. Replace the fuse block back into the voltage selector block
   Note: ensure that the correct voltage for your country is displayed.





Appendix 6 — Normalization theory

The importance of making a Normalization before initiating an experiment with any tubular tissue segment is described in Chapter x. In this appendix the mathematical rationale and calculations underlying the Normalization procedure are described in detail.

Mathematical calculations

Let (X_i, Y_i) be the pair of values representing the micrometer reading and force reading respectively characterising each step in the Normalization procedure. Y_0 is the force reading at the start position of the Normalization procedure where the wires are just separated and the force reading is approximately zero. Then, given that tension on the vessel is equal to force divided by wall length, the wall tension at the i-th micrometer reading is calculated by:

$$T_i = \frac{(Y_i - Y_0)}{2\delta \bullet (a_1 - a_2)}$$

where δ is the microscope eyepiece reticule calibration factor in mm per division and a_1 and a_2 are the vessel end points when measuring the length of the mounted vessel segment.

The internal circumference of the mounted vessel at the i-th reading is calculated by:

$$IC_i = IC_0 + (2 \bullet (X_i - X_0))$$

where IC_0 is the internal circumference of the mounted vessel when the wires are just separated and is given by:

$$IC_0 = (2 + \pi) \bullet d$$

where d is the wire diameter. For 40 µm wires, IC_0 = 205.6 µm.

Using the Laplace relation, the effective pressure P_i is calculated for each pair of readings. The effective pressure is an estimate of the internal pressure, which is necessary to extend the vessel to the measured internal circumference.

$$P_i = \frac{T_i}{\left(\frac{IC_i}{2\pi}\right)}$$

The stepwise distension is continued until the calculated effective pressure exceeds the target transmural pressure. The target value needs to be optimised for the individual tissue preparation (optimal active force as determined by the length-tension relationship for that tissue). For rat mesenteric arteries the target transmural pressure is normally 100 mmHg (13.3 kPa):

$$T_{100\ mmHg} = 100\ mmHg \bullet \left(\frac{IC}{2\pi}\right)$$

An exponential curve is fitted to the internal circumference pressure data as illustrated in fig. x in Chapter x. Now the isobar corresponding to 100 mmHg is used to calculate the IC_{100} value from the point of interception between the function of the exponential curve and the function of the 100 mmHg isobar.

The normalised internal circumference IC_1 is calculated by multiplying the internal circumference corresponding to 100 mmHg, IC_{100}, by a factor k. The factor is for rat mesenteric arteries 0.9. Again, this value should be optimised for the particular tissue preparation being used by a length-tension curve.

$$IC_1 = k \bullet IC_{100}$$

The normalised internal (lumen) diameter is then calculated by:

$$d_1 = \frac{IC_1}{\pi}$$

The micrometer reading X_1 at which the internal circumference of the normalised vessel is set to is calculated by:

$$X_1 = X_0 + \frac{(IC_1 - IC_0)}{2}$$

Appendix 7 — Calibration of eyepiece reticule

Principles of ocular calibration

The purpose of calibrating the eyepiece reticule is to determine a conversion factor (δ) allowing the microscope to be used for measuring vessel segment lengths mounted in the wire myograph.

Several types of eyepiece reticules are available for such a purpose. The most simple and yet very useful type is a horizontal scale as illustrated in fig. A7.1.

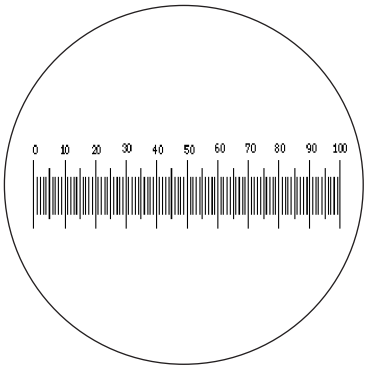


Figure A7.1 *Horizontal eyepiece reticule scale*

The basic principle is to use the eyepiece reticule, typically consisting of 50-100 divisions, to measure the length of an object in terms of reticular divisions spanned by the object. Having the conversion factor specific for the eyepiece reticule and used magnification, the length of the object in mm is easily calculated.

All reticules need to be calibrated in order to determine the specific conversion factor characteristic for the particular eyepiece reticule and the used magnification. For such purpose a special devise called a stage micrometer is needed. A stage micrometer is simply a microscope glass slide having a scale engraved on the surface. A typical micrometer scale is 2.00 mm long engraved with divisions of 0.01 mm equalling 10µm per division. However micrometer glass slides with less fine divisions are also useful for calibrating a stereomicroscope to be used with the wire myograph.

Ocular calibration procedure

1) Decide which microscope magnification is to be used for the segment length measurements. Use the largest possible fixed magnification where the eyepiece reticule scale still covers the whole gap of the myograph jaws.

2) Place the stage micrometer on the microscope stage and focus on it. Fit one of the division lines on the stage micrometer to one of the division lines of the reticule scale very precisely. While keeping the stage micrometer absolutely fixed on the microscope stage, find another position on both scales where the division lines also fit precisely. Read the position of the two “fit points” on both scales and fill in the values in the Ocular calibration sheet. Repeat the procedure twice.

Ocular Calibration Sheet

Date:	**Operator:**
Microscope (type and #):	
Ocular type:	
Magnification:	**Front lens:**

	Stage micrometer			Reticular scale		
Measurement	Position 1	Position 2	Length	Position 1	Position 2	Length
1	div.	div.	mm	div.	div.	div.
2	div.	div.	mm	div.	div.	div.
3	div.	div.	mm	div.	div.	div.
Mean			mm			div.

Calculations

1) Calculate the length between the two positions on the stage micrometer by subtracting the value of position 1 from the value of position 2. Dualply the length in divisions with the length of each division to get the length in mm.

2) Calculate the length between the two positions on the reticule scale by subtracting the value of position 1 from the value of position 2.

3) Calculate the mean length value of both the stage micrometer and the reticular scale.

4) Calculate the conversion factor:

$$\delta = \frac{\text{Mean Stage Micrometer Length (mm)}}{\text{Mean Reticule Scale (div)}}$$

Appendix 8 — How to read a millimetre micrometer

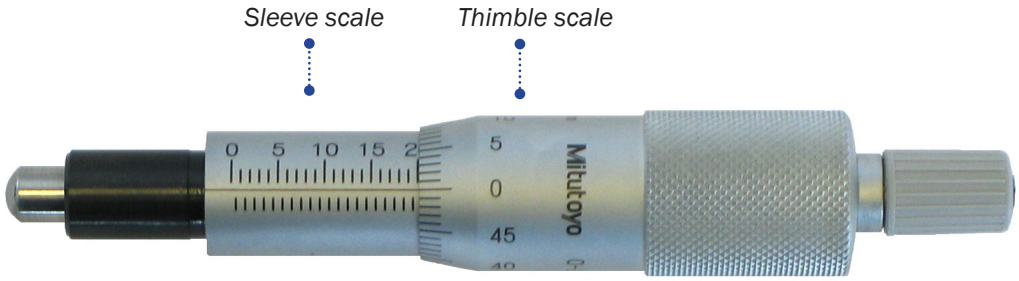


Figure A7.1 *Overview of the micrometer parts (actual reading 20000 µm = 20 mm)*

Sleeve scale
The micrometer sleeve scale has a total length of 25 mm divided into fifty equal parts. Each part of division above the horizontal line represents 1 mm where each fifth line is marked by a longer line and a number, which designates the length in mm. Each part of division below the horizontal line is placed between each 1 mm mark (scale above the horizontal line) and represents 0.5 mm.

Thimble scale
The thimble is divided into fifty equal parts, and one complete rotation of the thimble is indicated by the smallest division on the sleeve, which equals 0.5 mm. Each division on the thimble scale is 10 µm. If the thimble scale falls between two lines, then a number between 0 and 10 µm must be approximated.

Example 1

1. Note that the thimble has stopped at a point beyond "10" on the sleeve indicating 10000 µm (10 mm).
2. Note that there is no mark completely visible between the 10 mm mark and the thimble.
3. Read the value on the thimble corresponding to the intersection with the horizontal line on the sleeve.

A.	Reading on sleeve	10000 µm
B.	No additional mark visible	0 µm
C.	Thimble reading	380 µm
	Total reading	**10380 µm**

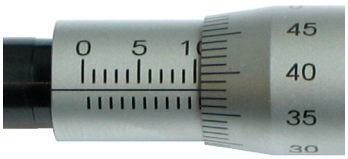


Figure A7.2 *Example 1: reading = 10380 µm*

Example 2

1. Note that the thimble has stopped at a point beyond "16" on the sleeve indicating 16000 µm (16 mm).
2. Note that this time a mark is visible between the 16 mm mark and the thimble indication 500 µm.
3. Read the value on the thimble corresponding to the intersection with the horizontal line on the sleeve.

A.	Reading on sleeve	16000 µm
B.	One additional mark visible	500 µm
C.	Thimble reading	280 µm
	Total reading	**16780 µm**

Figure A7.3*. Example 2: reading = 16780 µm*

Appendix 9 - System specifications

Tissue size:	>60µm
Chamber:	Auto Dual chambers
Chamber material:	Acid-resistant stainless steel
Chamber volume:	Max. 10ml
Chamber gassing:	Yes
Chamber cover:	Supplied with connections for gassing
Force range:	-100 -/+200mN
Force resolution:	0.01mN
Micropositioners:	Automatical linear motor operated
Weight calibration:	Manual
Heating:	Built-in
Temp. range:	Ambient temp. - 50°C
Temp. resolution:	0.1°C
Temp. probe:	External
Output reading:	Force (mN)
Analogue output:	Up to four output, 1.0V full scale for all acquired signals, user de fined
Digital output:	Serial interface - RS232 / RS485
Voltage:	100 to 240 VAC (auto) 50/60Hz
Ambient temp.	15-30° C

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